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MEDICINAL CHEMISTRY RESEARCH

ORIGINAL RESEARCH

Diazabenzo[a]phenoxazone sulphonamides: synthesis, in-silico and in-vitro antimicrobial studies

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Abstract

The syntheses of new sulphonamide derivatives of 8,10-diazabenzo[a]phenoxazones are reported. The condensation of 4,5-diamino-6-hydroxy-2-mercaptopyrimidine and 2,3-dichloro-1,4-naphthoquinone in a basic medium gave the key functional intermediate, 11-amino-6-chloro-9-mercapto-8,10-diazabenzo[a]phenoxazin-5-one. The conversion of the later compound to its sulphonamide derivatives was achieved via nickel catalyzed cross-coupling Buchwald-Hartwig protocol. Reaction between 11-amino-6-chloro-9-mercapto-8,10-diazabenzo[a]phenoxazin-5-one and various aryl sulphonamides and sulphonyl chlorides furnished eight new mono sulphonamide substituted diazaphenoxazone compounds. Subsequent coupling of mono sulphonamide substituted diazaphenoxazone compounds **5a-d** with four different arylsulphonyl chlorides under similar reaction conditions gave the disubstituted derivatives **8a-d**. The products were isolated in 74 – 88% yields and characterized by means of Uv-visible, FT-IR, ^{1}H-NMR, ^{13}C-NMR, and Mass spectroscopy. The synthesized compounds were screened for antimicrobial activity against bacterial strains: *Bacillus subtilis, Staphylococcus aureus, Escherichia coli, Salmonella typhi* and *Klebsiella pneumonia, and fungal strains, Aspergillus niger, and Candida albican*, using agar-well diffusion method. The activities of the compounds were compared with that of colymycin, which is a strong antibacterial, and antifungal drug, Most of the compounds showed appreciable antimicrobial activities comparable with the activity of colymycin. The i*n silico* study revealed that all the synthesized compounds showed significant binding affinity for both intact and mutated DNA gyrase. Compounds 8a and 5b showed the highest binding affinities of -12.31 and -13.30 kcal/mol for intact and mutated DNA gyrase respectively.

Keywords Sulphonamide · Diazaphenoxazone · Antimicrobial activity · Nickel catalysis · DNA gyrase

Introduction

The field of Medicinal Chemistry has continued to witness unabated interest for novel chemotherapeutic drugs because of the increasing resistance of bacterial and fungal microbes against existing clinical drugs (Aarestrup 2009; Threlfall et al. 2009). This necessitates a purposeful search for novel chemical moieties that can effectively handle these diseases. Bacterial DNA gyrase (Fig. 1) has been established as a drug target for the development of new antibiotics (Hooper 1998 and Maxwell 1997). It is a type II DNA topoisomerase that catalyses changes in the topology of DNA (Bates and Maxwell 2005; Wang 2009). Essentially, this enzyme is very crucial for almost all cellular processes, including replication, recombination and transcription in DNA.

Phenoxazines have been discovered to inhibit DNA topoisomerase. The phenoxazine compound represented as A in Fig. 2 has been shown to exhibit a low micro molar inhibitory activity (IC50 = 0.8–2.0 μM) against Escherichia coli topoisomerase I (Yu et al. 2017). Compounds B & C in Fig. 2 have been reported as antibacterial agents (Rádl and Zikán 1989, Chu and Maleczka 1987). In fact, the pharmacological activities of phenoxazines are well known and numerous.

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They are used as antitumor (Shimamoto et al. 2001, Harton et al. 1993), antituberculosis (Boothroyd and Clark 1952), and antibacterial (Chu Daniel 1986) agents among others. A water soluble 2-amino-4,4α,7-dimethyl-3*H*-phenoxazine was reported to possess antiproliferative, immunosuppressive, antibacterial and antiviral effects (Onoabedje et al. 2016). Natural products containing phenoxazine core such as actinomycin D, an antibiotic produced by Streptomyces, and others exhibit anticancer, antiviral and antifugal properties (Barness et al. 2015). More recently, Wang and his group isolated actinomycins A and B bearing 5H-oxazolo[4,5-b] phenoxazine chromophore from marine derived Streptomyces species. Actinomycins A showed potent cytotoxic activities against human cancer cell lines in the nanomolar range and moderate antibacterial activities against methicillin-resistant *Staphylococcus aureus* and vancomycin-resistant *Enterococci* strains (Wang et al. 2017).

On the other hand the sulphonamides have several applications especially as drugs. Sulphonamide drugs act by a competitive inhibition of *p*-aminobenzoic acid (PABA) use in the synthesis of dihydrofolate, which bacteria need to make DNA (Alovero et al. 1998). The sulphonamide drugs are synthetic antimicrobial agents that have broad spectrum of use with respect to Gram-positive and Gram-negative microorganisms (Vardayan and Hruby 2006). They are one of the most widely used antibacterial drugs in the world, particularly because of their low cost, low toxicity and excellent activity against common bacterial diseases (Ozbek et al. 2007). Many sulphonamide derivatives have been reported as carbonic anhydrase inhibitor (Zimmerman et al. 2004), anticancer (Scozzafava et al. 2003), anti- inflammatory agents (Weber et al. 2004), antibacterial (Eshghi et al. 2011), anti- HIV (Miller et al. 2002), antimalarial (Alqasoumi et al. 2010), antitumor (Chen et al. 2010), and antiviral agents (Rostom 2006). Some of the sulphonamide drugs currently available in the market include Bosentan (Ueda et al. 2011) (an anti hypertensive agent), Amprenavir (Shen et al. 2010 (an antiviral HIV protease inhibitor), Sildenafil (a phosphodiasterase-5 inhibitor) (Setter et al. 2005), Glibenclamide (Marble 1971) (an antidiabetic drug), Glimepriride (Marble 1971) (an antidiabetic nonantibiotic) and Torasemide ((Lopez et al. 2009)(the diuretic drug).

The wide range of medicinal and industrial applications of phenoxazine and sulphonamide compounds have led to continued synthesis of phenoxazine and sulphonamide derivatives (Ugwu et al. 2014; Onoabedje et al. 2016; Ibeanu et al. 2018). Therefore, we described the synthesis and the *in vitro* antimicrobial screening of diazabenzo[a]phenoxaxine derivatives incorporating sulphonamide and phenoxazine moieties. The synthesized compounds were subjected to *in-silico* studies in order to evaluate their drug-likeness. Their molecular docking using DNA gyrase, was also carried out to determine their binding affinities with the receptors. We also went further to mutate the DNA gyrase with a view to determine how effective the compounds can tackle resistant strains.

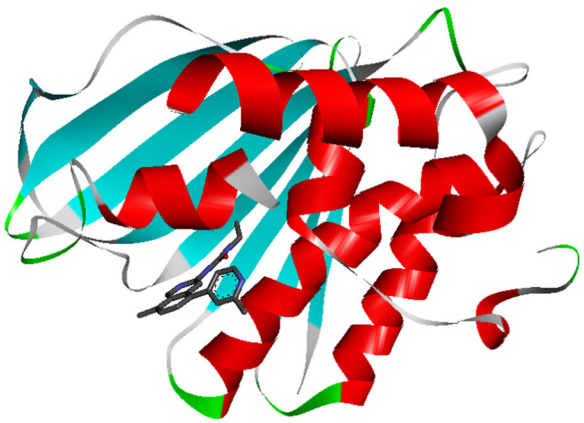


Fig. 1 3D ribbon representation of DNA gyrase with the co-crystallized ligand (1-Ethyl-3-[8-methyl-5-(2-methylpyridin-4-yl) isoquinolin-3-yl]urea)

EXPERIMENTAL SECTION

General

The reagents used were of analytical grades and were products of Sigma-Aldrich chemicals. Melting points were determined using electrothermal melting point apparatus in open capillaries and were uncorrected. Ultraviolet and visible spectra were recorded on UV-25500 PC spectrophotometer using matched 1 cm quart cell; absorption maxima were given in nanometers (nm), while the figures in parenthesis were the log of molar absorptivity coefficient

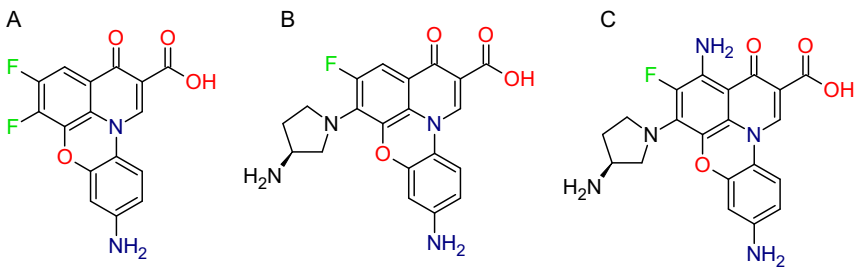


Fig. 2 Chemical structures of phenoxazine derivatives that are DNA topoisomerase inhibitors

(ε). The IR spectra were recorded on 8400 S FTIR spectrometer using KBr discs. The ^{1}H-NMR and ^{13}C-NMR were determined using varian NMR 400 MHZ spectrometer at Old Core Laboratory, Indian Institute of Technology, Pradesh, India; chemical shifts reported in (δ-ppm) scale. The antimicrobial screening was done at the Faculty of Pharmaceutical Sciences, University of Nigeria, Nsukka. All the products were purified through repeated recrystallization using suitable solvent(s). Bis(triphenylphosphine)nickel(II)chloride was synthesized according to literature procedure (Venanzi 1958). The physicochemical properties used for the evaluation of the drug-likeness of the synthesized compounds were calculated using the molecular descriptors available in the online open access molinspiration (www.molinspiration.com). The molecular descriptors calculated include: molecular weight (MW), partition coefficient (log P), hydrogen bond acceptor (HBA), hydrogen bond donor (HBD), topological polar surface area (TPSA) and number of rotatable bond (NoRB). We retrieved the crystal structure of DNA gyrase (PDB codes: 5MMN) containing the co-crystallized inhibitor from the online protein data bank repository (https://www.rcsb.org/structure/5mmn). Further preparations of the protein and the ligands were done using the discovery studio. These preparations included deleting of multiple chains, water of crystallization from the protein and energy minimization of the structures. The molecular docking and the visualization of the protein-ligand complex interaction were done using Autodock/Autodock vina and discovery studio respectively.

Synthesis of 11-amino-6-chloro-9-mercapto-8,10-diazabenzo[a]phenoxazin-5-one (3)

Into a 250 ml three-necked round bottom flask equipped with magnetic stirrer bar and a reflux condenser was added 4,5-diamino-6-hydroxy-2-mercaptopyrimidine **1** (2.50 g, 16 mmol) and anhydrous sodium carbonate (2.50 g, 23 mmol). A mixture of benzene (120 ml) and DMF (15 ml) was added and the mixture stirred for 45 min while heating on a water bath at 75–80 °C. 2,3-Dichloro-1,4-naphthoquinone **2** (3.60 g, 15 mmol) was then added and the resulting mixture was refluxed with continuous stirring for 5 h in a water bath at 75–80 °C. Benzene was distilled off and the slurry poured into a beaker containing distilled water (100 ml) and stirred to dissolve the inorganic materials. The mixture was allowed to cool overnight, filtered and the residue recrystallized from equal mixture of acetone and diethyl ether to give an intense reddish-brown crystalline solid of compound **3**. Yield 4.25 g, (81%), m.p. 195–197 °C. Uv-Vis (MeOH) (nm): 330 (2.5797), 450 (2.3424), 630 (1.7781), 752 (1.6021). IR (KBr) (cm^{-1}): 3510 (NH), 3140 (CH aromatic), 2507 (SH), 1723 (C=O), 1623 (C=N, C=C aromatic), 849 (C-Cl). ^{1}H-NMR (DMSO-d_6) δ: 7.89–7.52 (4H, m, Ar-H), 6.84 (2H, s, NH_2), 3.42 (1H, s, SH). ^{13}C-NMR (DMSO-d_6) δ: 186.42 (CO), 134.36, 134.13, 132.29, 131.31, 130.21, 127.43, 127.02, 126.37, 126.17, 124.70 and 124.39 (aromatic carbons). MS: in m/z [rel.%]: 315.00 [m^+ - NH_2, 27%], 313.07 [m^+ - O, 48%], 301.13 [m^+ - SH, 100%], 295.00 [m^+ - Cl, 33%].

General procedure for the synthesis of monosubstituted derivatives (5a-d and 7a-d)

To a mixture of bis(triphenylphosphine) nickel (II) chloride (3.00 g, 5 mmol) and triphenylphosphine (1.85 g, 10 mmol) was added t-butanol (6 ml) and distilled water (3 ml) with continuous stirring for 10 min at room temperature under nitrogen atmosphere. The resultant mixture was heated at 80 °C in an oil bath for 1.5 min. To the resultant mixture was added a mixture of 11-amino-6-chloro-9-mercapto-8,10-diazabenzo[a]phenoxazin-5-one **8** (2.50 g, 8 mmol), K_2CO_3 (1.38 g, 10 mmol) and the aryl sulphonamides or sulphonyl chlorides (1.80 g) in the solvents (t-butanol and distilled water in the ratio of 2:1). The mixture was refluxed at 100–110 °C under nitrogen atmosphere for 1 h with continuous stirring. The mixture was allowed to cool, filtered via suction, and washed with distilled water. The crude product was recrystallized from equal mixture of acetone and diethyl ether to give derivatives **11a-d** and **12a-d** respectively, in good to excellent yields.

11-Amino-6-(phenylsulphonamido)-9-mercapto-8,10-diazabenzo[a]phenoxazin-5-one (5a)

11-Amino-6-chloro-9-mercapto-8,10-diazabenzo[a]phenoxazin-5-one and benzene sulphonamide (1.80 g, 11 mmol) were converted to the title compound **5a** as a dark brown crystalline solid. Yield 2.66 g (78 %), mp. 206–208 °C. UV-Vis (MeOH) (nm): 330 (2.0791), 446 (2.000), 639 (1.7781). IR (KBr) (cm^{-1}): 3505, 3369 (NH), 3128 (CH aromatic), 2556 (SH), 1774 (C=O), 1619 (C=N), 1447 (C=C aromatic), 1277, 1134 (SO_2 two bands). ^{1}H-NMR (DMSO-d_6) δ: 7.68 (1H, s, NH), 7.66 (1H, d, Ar-H), 7.65 (1H, d, Ar-H), 7.63 (m, 1H, Ar-H), 7.55–7.46 (m, 4H, Ar-H), 7.24 (s, 2H, NH_2), 1.58 (s, 1H, SH). ^{13}C-NMR (DMSO-d_6) δ: 134.92, 133.92, 133.74, 132.23, 132.14, 132.05, 128.80, 128.66, 128.61, 128.54, 119.11 (aromatic carbons).

11-Amino-6-(4-methylphenylsulphonamido)-9-mercapto-8,10-diazabenzo[a]phenoxazin-5-one (5b)

11-Amino-6-chloro-9-mercapto-8,10-diazabenzo[a]phenoxazin-5-one and p-toluene sulphonamide (1.0 g, 10 mmol) were converted to the title compound **5b** furnished as a

brownish crystalline solid. Yield 3.01 g (87%), mp. 212–214 °C. UV-Vis (MeOH) (nm): 330 (2.1271), 440 (1.9031), 651 (1.4771). IR (KBr) (cm^{-1}): 3533, 3336, (NH), 3099, (CH aromatic), 2509 (SH), 1700 (C=O), 1628, (C=N), 1475 (C=C), 1293, 1125 (SO_2 two bands). ^{1}H-NMR (DMSO-d_6) δ: 7.67 (1H, s, NH), 7.65 (1H, d, Ar-H), 7.62 (1H, d, Ar-H), 7.53–7.44 (4H, m, Ar-H), 7.24 (2H, s, NH_2), 2.41 (1H, s, SH), 1.63 (s, 3H, Ar-CH_3). ^{13}C-NMR (DMSO-d_6) δ: 177.14 (CO), 139.72, 132.23, 132.12, 132.04, 128.65, 128.52, 126.37, 116.50 (aromatic carbons), 35.02 (CH_3). MS: in m/z [rel.%]: 295.13 [m^+ - $PhCH_3NHSO_2^+$, 100%], 267.13[m^+ - $PhCH_3NHSO_2^+$ SH, 9%], 261.27 [m^+ - $PhCH_3NHSO_2^+$ NH_2, 4%], 155.00 [13%], 152.33 [2%].

11-Amino-6-(4-chlorophenylsulphonamido)-9-mercapto-8,10-diazabenzo[a]phenoxazin-5-one (5c)

11-Amino-6-chloro-9-mercapto-8,10-diazabenzo[a]phenoxazin-5-one and 4-chlorobenzene sulphonamide (1.80 g, 9 mmol) were converted to the title compound **5c** obtained as a deep brown crystalline solid. Yield 3.05 g, (83%), mp. 216–218 °C. UV-Vis (MeOH) (nm): 330 (2.3010), 448 (1.9030), 648 (1.6020). IR (KBr) (cm^{-1}): 3507, 3349 (NH), 3119 (aromatic CH), 2573 (SH), 1734 (C=O), 1574 (C=N), 1498 (C=C aromatic), 1231, 1128 (SO_2 two bands), 841 (C-Cl). ^{1}H-NMR (DMSO-d_6) δ: 7.77 (1H, s, NH), 7.56 (1H, d, Ar-H), 7.50 (1H, d, Ar-H), 7.34 (4H, m, Ar-H), 7.18 (2H, s, NH_2), 3.29 (1H, s, SH). ^{13}C-NMR (DMSO-d_6) δ: 195.80 (CO), 154.09, 137.17, 133.82, 133.69, 132.58, 132.37, 132.04, 131.95, 129.52, 129.33, 129.25 (aromatic carbons). MS: in m/z [rel.%]: 297.07 [m^+ - $PhClNHSO_2^+$, 12%], 297.33 [m^+ - $PhClNHSO_2^+$ NH_2, 37%], 245.00 [m^+ - $PhClNHSO_2^+$ SH, 11%], 152.93 [6%].

11-Amino-6-(4-nitrophenylsulphonamido)-9-mercapto-8,10-diazabenzo[a]phenoxazin-5-one (5d)

11-Amino-6-chloro-9-mercapto-8,10-diazabenzo[a]phenoxazin-5-one and 4-nitrobenzene sulphonamide (1.80 g, 9 mmol) were converted to the title compound **5d** furnished as a pale brownish crystalline solid. Yield 3.11 g, (83%), mp. 222–224 °C. UV-Vis (MeOH) (nm): 330 (2.5797), 651 (1.6232). IR (KBr) (cm^{-1}): 3567, 3337 (NH), 3070 (CH aromatic), 2586 (SH), 1739 (C=O), 1568 (C=N), 1407 (C=C aromatic), 1277 (N=O), 1159, 1078 (SO_2 two bands). ^{1}H-NMR (DMSO-d_6) δ: 8.20 (1H, s, NH), 7.57 (1H, d, Ar-H), 7.51 (1H, d, Ar-H), 7.28–7.11 (4H, m, Ar-H), 5.77 (2H, s, NH_2), 3.29 (1H, s, SH). ^{13}C-NMR (DMSO-d_6) δ: 191.59 (CO), 152.93, 133.71, 132.70, 132.55, 132.32, 132.01, 131.92, 129.31, 129.21 (aromatic carbons). MS: in m/z [rel.%]: 291.83 [m^+ - $PhNO_2NHSO_2^+$, 10%], 277.53 [m^+ - $PhNO_2NHSO_2^+$ NH_2, 4%], 187.87 [5%], 156.33 [4%].

11-(Phenylsulphonamido)-6-chloro-9-mercapto-8,10-diazabenzo[a]phenoxazin-5-one (7a)

11-Amino-6-chloro-9-mercapto-8,10-diazabenzo[a]phenoxazin-5-one and benzene sulphonylchloride (1.80 g, 10 mmol) were converted to the title compound **7a** as a dark reddish crystalline solid. Yield 2.70 g (76%), mp. 210–212 °C. UV-Vis (MeOH) (nm): 330 (2.5797), 550 (2.5563), 692 (1.9031). IR (KBr) (cm^{-1}): 3361, 3242 (NH), 3127 (CH aromatic), 2489 (SH), 1653 (C=O, C=N), 1472 (C=C aromatic), 1231, 1102 (SO_2 two bands), 868 (C-Cl). ^{1}H-NMR (DMSO-d_6) δ: 8.79 (1H, s, NH), 7.59 (1H, d, Ar-H), 7.57 (1H, d, Ar-H), 7.54 (1H, m, Ar-H), 7.51–7.26 (4H, m, Ar-H), 3.45 (1H, s, SH). ^{13}C-NMR (DMSO-d_6) δ: 173.72 (CO), 153.72, 132.59, 132.05, 131.95, 129.35, 129.23, 128.95, 128.18, 127.74 (aromatic carbons).

11-(4-Methylphenylsulphonamido)-6-chloro-9-mercapto-8,10-diazabenzo[a]phenoxazin-5-one (7b)

11-Amino-6-chloro-9-mercapto-8,10-diazabenzo[a]phenoxazin-5-one and p-toluenesulphonylchloride (1.80 g, 9 mmol) were coupled to obtain the title compound **7b** furnished as a brownish crystalline solid. Yield 3.15 g (86%), mp. 218–220 °C. UV-Vis (MeOH) (nm): 330 (2.2041), 460 (1.7781), 600 (1.3010), 692 (1.4771). IR (KBr) (cm^{-1}): 3251 (NH), 3115 (CH aromatic), 2491 (SH), 1639 (C=O), 1510 (C=N), 1438 (C=C aromatic), 1123, 1076 (SO_2 two bands), 897 (C-Cl). ^{1}H-NMR (DMSO-d_6) δ: 7.85 (1H, s, NH), 7.50 (4H, m, Ar-H), 7.12 (4H, m, Ar-H), 3.32 (1H, s, SH), 2.20 (3H, s, Ar-CH_3). ^{13}C-NMR (DMSO-d_6) δ: 133.65, 132.57, 132.01, 131.94, 129.31, 129.22 (aromatic carbons), 38.97 (CH_3).

11-(4-Chlorophenylsulphonamido)-6-chloro-9-mercapto-8,10-diazabenzo[a]phenoxazin-5-one (7c)

11-Amino-6-chloro-9-mercapto-8,10-diazabenzo[a]phenoxazin-5-one and 4-chlorobenzenesulphonylchloride (1.80 g, 8 mmol) were converted to the title compound **7c** was furnished as a greyish crystalline solid. Yield 3.29 g (86%), mp. 219–221 °C. UV-Vis (MeOH) (nm): 330 (2.3873), 453 (2.1461), 690 (1.7781). IR (KBr) (cm^{-1}): 3334 (NH), 3193 (CH aromatic), 2331 (SH), 1667 (C=O), 1545 (C=N, C=C aromatic), 1220, 1137 (SO_2 two bands), 879 (C-Cl). ^{1}H-NMR (DMSO-d_6) δ: 7.69 (1H, s, NH), 7.49 (4H, m, Ar-H), 3.28 (1H, s, SH). ^{13}C-NMR (DMSO-d_6 + $CDCl_3$) δ: 132.48, 131.99, 131.93, 129.16, 129.07 (aromatic carbons). MS: in m/z [rel.%]: 315.13 [m^+ - $PhClNHSO_2^+$, 10%], 301.13 [m^+ - $PhClNHSO_2^+$ O, 100%], 297.33 [m^+ - $PhClNHSO_2^+$ Cl, 67%], 175.00 [21%], 153.27 [2%].

11-(4-Nitrophenylsulphonamido)-6-chloro-9-mercapto-8,10-diazabenzo[a]phenoxazin-5-one (7d)

11-Amino-6-chloro-9-mercapto-8,10-diazabenzo[a]phenoxazin-5-one reacted with 4-nitrobenzenesulphonylchloride (1.80 g, 8 mmol) to afford the title compound **7d** as a pale brownish crystalline solid. Yield 3.28 g (84 %), mp. 226–228 °C. UV-Vis (MeOH) (nm): 330 (2.5797), 504 (1.9031), 700 (1.6021). IR (KBr) (cm^{-1}): 3393 (NH), 3155 (CH aromatic), 2453 (SH), 1639 (C=O, C=N), 1469 (aromatic C=C), 1338 (N=O), 1165, 1012 (SO_2 two bands), 838 (C-Cl). ^{1}H-NMR (DMSO-d_6) δ: 8.18 (1H, s, NH), 7.84 (1H, d, Ar-H), 7.55 (1H, d, Ar-H), 7.51–7.11 (4H, m, Ar-H), 3.31 (1H, s, SH). ^{13}C-NMR (DMSO-d_6) δ: 133.68, 132.57, 132.01, 131.94, 129.31, 129.21, 125.22 (aromatic carbons). MS: in m/z [rel.%]: 316.93 [m^+ - $PhNO_2NHSO_2^+$, 7%], 301.13 [m^+ - $PhNO_2NHSO_2^+$ O, 100%], 297.33 [m^+ - $PhNO_2NHSO_2^+$ Cl, 35%], 185.93 [16%], 156.20 [3%].

General procedure for synthesis of disubstituted derivatives (8a–d)

To a mixture of bis(triphenylphosphine) nickel (II) chloride (3.00 g, 5 mmol) and triphenylphosphine (1.85 g, 10 mmol) was added t-butanol (6 ml) and distilled water (3 ml) with continuous stirring for 10 min at room temperature under nitrogen atmosphere. The resultant mixture was heated at 80 °C in an oil bath for 1.5 min, after which 11-amino-6-substituted-9-mercapto-8,10-diazabenzo[a]phenoxazin-5-one **8a-d** (2.50 g), K_2CO_3 (1.38 g, 10 mmol) and aryl sulfonylchlorides (1.80 g) were added to it with the help of solvents (t-butanol and distilled water in the ratio of 2:1). The mixture was refluxed at 100–110 °C under nitrogen atmosphere for 1 h with continuous stirring. At the end of the refluxing period, the mixture was allowed to cool, filtered via suction, and washed with distilled water. The crude product was recrystallized from equal mixture of acetone and diethyl ether to give derivatives **8a-d** in good to excellent yields.

Synthesis of 6,11-bis(phenylsulfonamido)-9-mercapto-8,10-diazabenzo[a]phenoxazin-5-one 8a

Compound **8a** was obtained as a reddish-yellow crystalline solid when 11-amino-6-(phenylsulfonamido)-9-mercapto-8,10-diazabenzo[a]phenoxazin-5-one **5a** (2.50 g, 5 mmol) was coupled with benzenesulfonylchloride (1.0 g, 10 mmol). Yield 2.43 g (74%), m.p. 230–232 °C. UV-Vis (MeOH) λ_{max}: 330 (2.5185), 640 (2.6628), 700 (2.8451) nm. FT-IR(KBr);V_{max}: 3293 (N-H stretch), 3043 (Ar C-H stretch), 2481, 2418 (S-H stretch), 1661 (C=O, C=N), 1591, 1421 (Ar-C=C), 1267, 1040 cm^{-1} (S=O). ^{1}H-NMR (DMSO:d_6) $\delta_{(ppm)}$: 7.60 (1H, s, NH), 7.58 (1H, d, Ar-H), 7.57 (1H, d, Ar-H), 7.52 (1H, m, Ar-H), 7.51 (4H, m, Ar-H), 3.38 (1H, s, SH). ^{13}C-NMR (DMSO) $\delta_{(ppm)}$: 136.66 (imine carbon), 134.97–127.63 (aromatic carbons).

Synthesis of 6,11-bis(4-methylphenylsulfonamido)-9-mercapto-8,10-diazabenzo[a]phenoxazin-5-one 8b

Compound **8b** was obtained as a brownish crystalline solid when 11-amino-6-(4-methylphenylsulfonamido)-9-mercapto-8,10-diazabenzo[a]phenoxazin-5-one **5b** (2.50 g, 5 mmol) reacted with p-toluenesulfonylchloride (1.80 g, 9 mmol). Yield 2.71 g (85%), m.p. 264–268 °C. Uv-Vis (MeOH) λ_{max}: 330 (2.5185), 460 (2.6628), 600 (2.7782) nm. FT-IR (KBr);V_{max}: 3261 (N-H stretch), 3190, 3110 (Ar C-H stretch), 2498 (S-H stretch), 1660 (C=O, C=N), 1585, 1420 (Ar-C=C), 1024 (S=O), 719 cm^{-1} (para substitution). ^{1}H-NMR (DMSO:d_6) $\delta_{(ppm)}$: 7.68 (1H, s, NH), 7.66 (1H, d, Ar-H), 7.59 (1H, d, Ar-H), 7.57 (4H, m, Ar-H), 3.35 (1H, s, SH), 2.03 (3H, s, CH_3). ^{13}C-NMR (DMSO) $\delta_{(ppm)}$: 142.40 (C=O), 141.93 (imine carbon), 133.70–126.13 (aromatic carbons), 39.06 (CH_3).

Synthesis of 6,11-bis(4-chlorophenylsulfonamido)-9-mercapto-8,10-diazabenzo[a]phenoxazin-5-one 8c

Compound **8c** was furnished as a dark reddish crystalline solid on coupling 11-amino-6-(4-chlorophenylsulfonamido)-9-mercapto-8,10-diazabenzo[a]phenoxazin-5-one **5c** (2.50 g, 5 mmol), with 4-chlorobenzenesulfonylchloride (1.80 g, 8 mmol). Yield 2.89 g (88%), m.p. 255–257 °C. Uv-Vis(MeOH) λ_{max}: 330 (2.5155), 520 (2.7160) nm. FT-IR(KBr);V_{max}: 3221 (N-H stretch), 3069 (Ar C-H stretch), 2501, 2413 (S-H stretch), 1715 cm^{-1} (C=O, C=N), 1582, 1456 (Ar-C=C), 1096 (S=O), 827, 678 cm^{-1} (C-Cl stretch, para substitution). ^{1}H-NMR (DMSO:d_6) $\delta_{(ppm)}$: 7.78 (1H, s, NH), 7.76 (1H, d, Ar-H), 7.59 (1H, d, Ar-H), 7.57 (4H, m, Ar-H), 3.31 (1H, s, SH). ^{13}C-NMR (DMSO) $\delta_{(ppm)}$: 167.34 (C=O), 158.96 (imine carbon), 145.77–110.04 (aromatic carbons).

Synthesis of 6,11-bis(4-nitrophenylsulfonamido)-9-mercapto-8,10-diazabenzo[a]phenoxazin-5-one 8d

Compound **8d** was obtained as a brownish crystalline solid when 11-amino-6-(4-nitrophenylsulfonamido)-9-mercapto-8,10-diazabenzo[a]phenoxazin-5-one **5d** (2.50 g, 5 mmol) was coupled with 4-nitrobenzenesulfonylchloride (1.80 g, 8 mmol). Yield 2.69 g (81%), m.p. 274–276 °C. Uv-Vis (MeOH) λ_{max}: 330 (2.5185), 680 (2.8325) nm. FT-IR(KBr); V_{max}: 3395 (N-H stretch), 3157 (Ar C-H stretch), 2592, 2507 (S-H stretch), 1642 (C=O, C=N), 1563, 1469 (Ar-

C=C), 1330 (N=O), 1250 (S=O), 693 cm^{-1} (para substitution). ^{1}H-NMR (DMSO:d_6 + $CDCl_3$) δ $_{(ppm)}$: 7.49 (1H, s, NH), 3.26 (1H, s, SH). ^{13}C-NMR (DMSO + $CDCl_3$) δ $_{(ppm)}$: 133.38 (imine carbon), 132.25–128.90 (aromatic carbons).

Antimicrobial Activity Assays

The general sensitivity testing and MICs of the sulphonamides were investigated against Bacillus subtilis, Staphylococcus aurues, Escherichia coli, Salmonella typhi, klebsiella pneumoniae, Aspergillus niger and Candida albican which are associated with skin, urinary and chronic obstructive pulmonary infections, as well as gastrointestinal tract damage and renal failure.

Sensitivity Test

Agar-well diffusion technique as described (Okorie 2005) was used to determine the antimicrobial sensitivity test of the phenoxazine intermediate and that of the sulphonamide derivatives. Sensitivity test agar plates were seeded with 0.1 ml of culture of each microorganism into its corresponding Petri-dish. The plates were allowed to set after which cups were made in each sector drawn on the backside of the bottom plate using marker. Using a pipette, each cup was filled with six drops of their corresponding antimicrobial agents in appropriate solvent at a concentration of 50 mg/ml. The plates were incubated at 37 °C for 24 h for bacteria and 48 h for fungi. The zones of inhibition produced after the period of incubation was measured. The procedure was repeated for the reference drug colomycin.

Minimum inhibitory concentration (MIC)

Agar-well diffusion method was used to determine the minimum inhibitory concentration (MIC) of the synthesized compounds (Adeniyi and Odelola 1996). Serial dilutions of the compounds were prepared from 50 mg/ml solution of the sulphonamide derivatives to give 50, 25, 12.5, 6.25 and 3.125 mg/ml. After dilution, the test solutions were added into their corresponding cups previously made in the molten agar, starting from the lowest concentration (3.125 mg/ml). This was followed by incubation at the appropriate incubation temperature and time. The resultant inhibition zones of diameter (IZD) were measured and the values subtracted from the diameter of the borer (8 mm) to give the inhibition zone diameter (IZD). The graph of IZD^2 against log of concentration was plotted for each plate and the antilogarithm of the intercept on x-axis gave the MIC. The procedure was also repeated for colomycin.

In silico studies

The physicochemical properties used for the evaluation of the drug-likeness of the synthesized compounds were calculated using the molecular descriptors available in the online open access molinspiration (www.molinspiration.com). The molecular descriptors calculated include: molecular weight (MW), partition coefficient (log P), hydrogen bond acceptor (HBA), hydrogen bond donor (HBD), topological polar surface area (TPSA) and number of rotatable bond (NoRB). We retrieved the crystal structure of DNA gyrase (PDB codes: 5MMN) containing the co-crystallized inhibitor from the online protein data bank repository (https://www.rcsb.org/structure/5mmn). The chemical structures of the synthesized compounds were drawn using ChemSketch. Further preparations of the protein and the ligands were done using the discovery studio. These preparations included deleting of multiple chains, water of crystallization from the protein and energy minimization of the structures. The molecular docking and the visualization of the protein-ligand complex interaction were done using Autodock/Autodock vina and discovery studio respectively.

RESULTS AND DISCUSSION

Chemistry

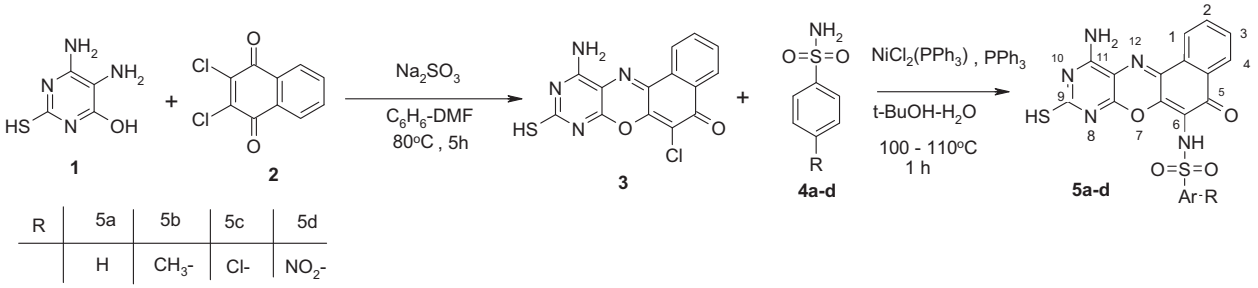
Anhydrous base catalyzed cross-coupling of 4,5-diamino-6-hydroxy-2-mercaptopyrimidine **1** and 2,3-dichloro-1,4-naphthoquinone **2** under refluxing condition for 5 h afforded the key intermediate, 11-amino-6-chloro-9-mercapto-8,10-diazabenzo[a]phenoxazin-5-one **3** (Scheme 1) (Okafor 1986). The UV-visible absorption bands of compound **3** gave 630 and 752 nm due to the presence of extensive pi-bond conjugation. The IR spectra of compound **3** revealed the presence of N-H stretching vibrations at 3510 cm^{-1} due to NH_2, while the weak band at 2507 cm^{-1} depicted the presence of S-H. There is also the presence of strong band at 1723 cm^{-1}, assigned to C=O, while the bands at 1623 and 1492 cm^{-1} are assigned to C=N and C=C aromatic respectively. The proton nuclear magnetic resonance of compound **3** furnished aromatic protons at δ 7.89–7.52, NH_2- at δ 6.84 and HS- at 3.42. The ^{13}C-NMR provided carbonyl carbon at 186.42, in addition to other carbon peaks. Compound **3** was converted into 6-aryl sulphonamidodiazaphenoxazinones **5a-d** via reactions with aryl sulphonamides **4a-d**.

Compounds **4a-b** were obtained by reactions of aryl sulphonyl chlorides with ammonium hydroxide solution (Ugwu et al. 2014). In this reaction, the aryl sulphonyl chlorides were stirred in aqueous ammonium hydroxide for

5 min followed by addition of water with stirring for further 3 mins. Thereafter, the entire reaction mixture was heated at 60 °C for 5 min, and allowed to cool with chilling. The desired products were obtained after filtration and recrystallization from aqueous ethanol. Compounds **5a-d** were highly coloured and obtained in high yields. Their structures were confirmed by combination of spectroscopic and analytical data. IR spectra of compound **5a-d** showed N-H stretching vibrations at two bands in the range 3503–3567 cm^{-1} (assigned to NH_2), and 3369–3337 cm^{-1} (assigned to NH of $NHSO_2$). The appearance of the second N-H vibration at lower bands together with that of S=O vibrations at 1277–1012 cm^{-1} supports the formation of sulphonamide group in the derivatives. Furthermore, the continued presence of absorption bands at 2489–2453 cm^{-1} (S-H stretching) and the absence of absorption bands at about 480–400 cm^{-1} (S-S stretch) in derivatives **5a-d** means that there was no coupling between the mercapto group in compound **3** and the chloride (Cl) of the aryl sulphonyl chlorides (Scheme 2).

Furthermore, compound **3** reacted with 4-substituted aryl sulphonyl chlorides **6a-d** to furnish compounds **7a-d** in excellent isolated yields after recrystalisation from mixture of acetone and diethyl ether.

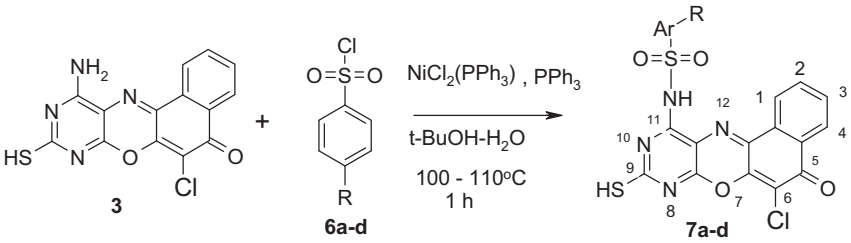
In another reaction, 4-substitued aryl sulphonyl chloride **6a-d** reacted with 11-amino-6-(sulphonamido)-9-mercapto-8,10-diazabenzo[a]phenoxazin-5-ones **5a-d** under nickel-phosphine catalytic system to furnish 6,11-bis(phenylsulfonamido)-9-mercapto-8,10-diazabenzo[a]phenoxazin-5-ones **8a-b** in high yields (Scheme 3). The structures



R	5a	5b	5c	5d
	H	CH_3-	Cl-	NO_2-

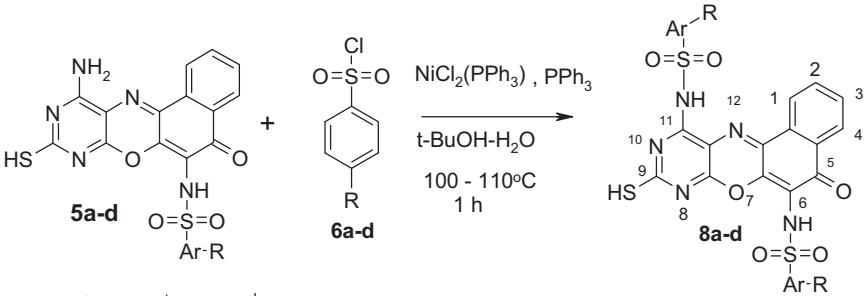
Scheme 1 The synthesis of 6-substituted aryl sulphonamide diazabenzo[a]phenoxazinones 5a-d

Scheme 2 Synthesis of 11-arylsulphonamidodiazabenzo[a]phenoxazinones **7a-d**



R	7a	7b	7c	7d
	H	CH_3-	Cl-	NO_2-

Scheme 3 Synthesis of 6,11-bisarylsulphonamidodiazabenzo[a]phenoxazinones **8a-d**



R	8a	8b	8c	8d
	H	CH_3-	Cl-	NO_2-

Table 1 Inhibition zone diameter (IZD) (mm) and MIC (mg/ml) in bracket ()

	Bacteria					Fungi	
Compd No	*B.subtilis*	*S.aureus*	*E.coli*	*S.typhi*	*K.pneumoniae*	*A.niger*	*C.albican*
3	14(0.53)	10(0.83)	12(0.80)	--	--	--	--
5a	--	--	--	--	--	--	--
5b	--	--	--	--	--	--	--
5c	12(0.90)	14(0.50)	--	--	--	--	--
5d	--	--	--	--	--	--	--
7a	18(0.26)	20(0.20)	22(0.12)	12(0.86)	16(0.28)	--	--
7b	19(0.24)	18(0.26)	16(0.28)	22(0.09)	14(0.36)	--	21(0.15)
7c	--	--	--	--	--	--	--
7d	--	--	--	--	--	--	--
8a	22(0.10)	12(0.78)	26(0.05)	15(0.30)	19(0.25)	13(0.81)	22(0.10)
8b	20	15	–	–	–	–	–
8c	10(0.92)	8(1.10)	–	–	–	–	14(0.83)
8d	--	--	--	–	--	–	–
Ref. Drug	23(0.09)	18(0.27)	19(0.24)	15(0.32)	21(0.14)	12(0.89)	17(0.29)

Reference drug = Colomycin

Table 2 Free binding energy of compounds with intact and mutated DNA gyrase

Comp	Intact DNA gyrase ΔG (kcal/mol)	Mutated DNA gyrase ΔG (kcal/mol)
3	−11.25	−11.59
5a	−11.46	−12.47
5b	−11.53	−13.30
5c	−11.43	−12.02
5d	−11.90	−12.67
7a	−10.94	−11.49
7b	−11.01	−12.56
7c	−11.76	−12.37
7d	−12.09	−12.43
8a	−12.31	−11.57
8b	−11.98	−11.13
8c	−11.62	−10.31
8d	−11.12	−11.69
Native ligand	−10.31	−12.07

Native ligand = 1-Ethyl-3-[8-methyl-5-(2-methylpyridin-4-yl) isoquinolin-3-yl]urea

were confirmed by combination of spectroscopic and analytical data.

Antimicrobial activity

The antimicrobial screening of the azaphenoxazone and its derivatives against *Bacillus subtilis, Staphylococcus aureus*, *Escherichis coli, Salmonella typhi, Klebsiella pneumoniae*, *Candida albican* and *Aspergillus niger* were carried out at the concentration of 50 mg/ml in agar media following the method described (Okorie 2005). Colymycin was used as reference drug for antibacterial and antifungal drugs. The activity of the compounds was measured in form of inhibition zone diameter (IZD) around the wells as well as minimum inhibitory concentration (MIC) and the results are presented in Table 1. Generally while some derivatives showed significant antimicrobial activity, others lost their activity completely on derivatization. Compounds **3**, **5c**, **7a**, **7b**, **8a**, **8b** and **8c** showed some degree of activity while compounds **5a**, **5b**, **5d**, **7c**, **7d**, **8d** showed no activity against any of the organisms tested. In particular compounds **7a**, **7b** and **8a** have broad spectrum of activity against Gram negative and Gram-positive bacteria. Specifically, both bacteria and fungi are susceptible to compound **8a** like the colomycin. Interestingly, compounds **7b** and **8a** have marked activity more than the standard drug at a very low dose against *S. Typhi* and *E. coli* respectively. Compounds **7b** and **8a** are therefore potential drugs for the treatment of infectious diseases like typhoid fever, food poisoning, gastroenteritis and enteric fever.

In silico studies

Lipinski's rule of five (ro5) was applied to adjudge the drug-likeness of the compounds. The ro5 implies that for a molecule to possess drug-likeness, the MW ≤ 500, HBD ≤ 5, HBA ≤ 10 and Log P ≤ 5 must be obeyed. If the molecule fails in more than one type of parameters, the molecule will probably have challenge in oral bioavailability. In Table 2, it can be seen that only **8c** failed in more than one whereas others passed. TPSA and NoRB are also very useful in assessing how useful a molecule can be as a drug. TPSA ≤

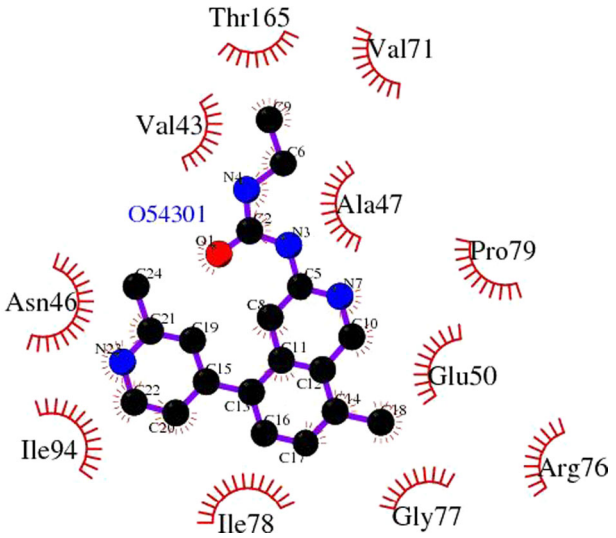


Fig. 3 Ligplot + of co-crystallized ligand (1-Ethyl-3-[8-methyl-5-(2-methylpyridin-4-yl) isoquinolin-3-yl]urea) in the binding cavity of intact DNA gyrase

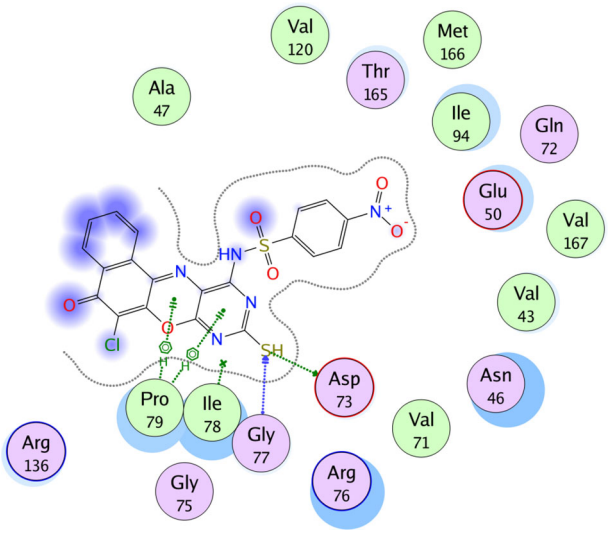


Fig. 4 Ligplot + of co-crystallized ligand (1-Ethyl-3-[8-methyl-5-(2-methylpyridin-4-yl) isoquinolin-3-yl]urea) in the binding cavity of mutated DNA gyrase

140 Å^2 and NoRB ≤ 10 would have a high probability of good oral bioavailability in rats (Veber et al. 2002).

Molecular docking

Mutation of DNA gyrase

The ligplot + of the intact and the mutated DNA gyrase are shown in Figs. 3 and 4 respectively. The mutation was done by replacing the GLU 42 and ASP 73 with ALA. Figure 4 shows clearly 12 active amino acid residues (THR 165, VAL 43, ALA 47, VAL 71, ASP 73, ASN 46, GLU 50, ILE 94, ARG 76, ILE 78, PRO 79 and GLY 77) in the intact DNA gyrase surrounding the co-crystallized ligand. H-bonding interactions between the ASP 76 and two different atoms of the co-crystallized ligand, in addition to other interactions were clearly shown. These were obviously absent in the mutated DNA gyrase. The ASP 76 was absent in the mutated gyrase as it has been replaced by ALA 76. There were observed, two pi-H interactions between the 6-membered ring of the ligand and the ILE 78 in the mutated enzyme.

Table 3 Physicochemical properties of the synthesized compounds

Comp	MW	logP(o/w)	NoRB	HBD	HBA	TPSA
3	330.755	1.863	0	2	4	121.16
5a	433.448	1.96	3	2	6	136.63
5b	465.514	2.816	3	2	6	175.43
5c	467.893	2.919	3	2	6	136.63
5d	478.445	2.262	4	2	6	182.45
7a	470.917	3.782	3	1	6	149.41
7b	484.944	4.08	3	1	6	149.41
7c	505.362	4.374	3	1	6	149.41
7d	515.914	3.717	4	1	6	195.23
8a	591.649	3.939	6	2	8	195.58
8b	619.703	4.535	6	2	8	195.58
8c	660.539	5.123	6	2	8	195.58
8d	681.643	3.809	8	2	8	287.22
Native ligand	320.396	2.931	5	2	3	66.91

Gross et al., in their extensive work on the active-site residues of *Escherichia coli* DNA gyrase observed that substitution of GLU 42 and ASP 73 with ALA leads to microbial resistance to some conventional antibiotics (Gross et al. 2003). The synthesized compounds were docked into the binding cavity of the DNA gyrase and the free binding energy was calculated. The DNA gyrase were mutated and the compounds were docked into the mutated target and the results are shown in Table 3.

The results as shown in Table 3 revealed that the synthesized compounds interacted significantly both with the intact and mutated DNA gyrase. There was no significant difference in the binding affinity of all the synthesized compounds. However, their binding energies are lower (high binding affinities) than the reference (native ligand) for the intact drug target. Compounds 8a and 5b have been used to show the binding interaction of the synthesized compounds with the both intact and mutated DNA gyrase respectively. The 2D interaction of **8a** with the intact gyrase has been shown in Fig. 5 while Fig. 6 shows the 2D interaction of compound 5b with mutated DNA gyrase.

Moreover, the compounds also showed significant binding affinity with the mutated DNA gyrase. The 6-substitued aryl sulphonamide diazabenzo[a]phenoxazones

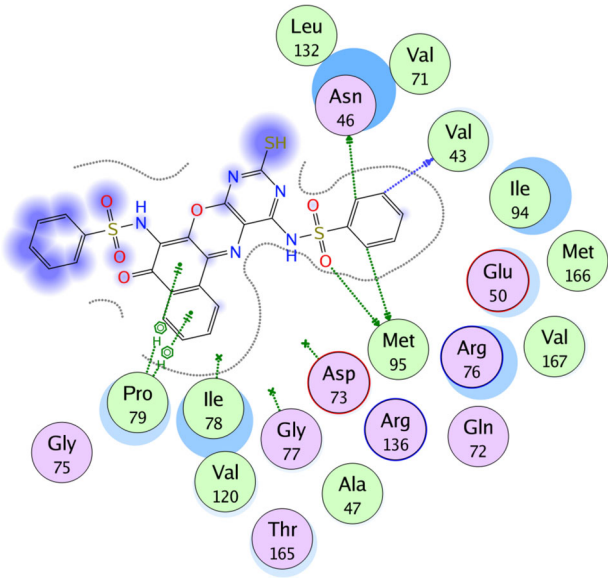


Fig. 5 2D interaction of compound 8a with intact DNA gyrase

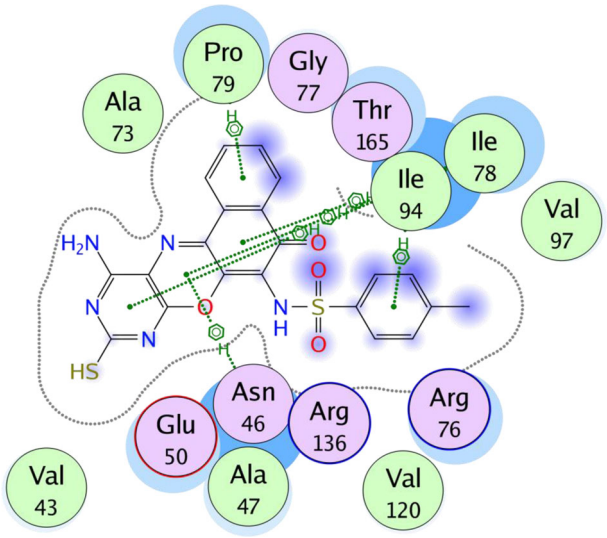


Fig. 6 2D interaction of compound 5b with mutated DNA gyrase

(5a-d) showed a better binding affinity with the mutated DNA gyrase than the rest of the compounds. Compound **5b** had the highest binding affinity as depicted by its lowest binding energy (−13.30 kcal/mol). It can be deduced that these 6-substitued aryl sulphonamide diazabenzo[a]phenoxazones may be more effective in the treatment of infections caused by resistant strains.

A closer analysis of the binding of **8a** with intact 5MMN (Figs. 5 and 7) showed various chemical interaction between the compound and the receptor. The amino acid residues: VAL43, ILE78, ILE94, PRO79, VAL120 and VAL167 showed pi-alkyl interactions with the aromatic rings. There were also amide-pi sacked interactions between

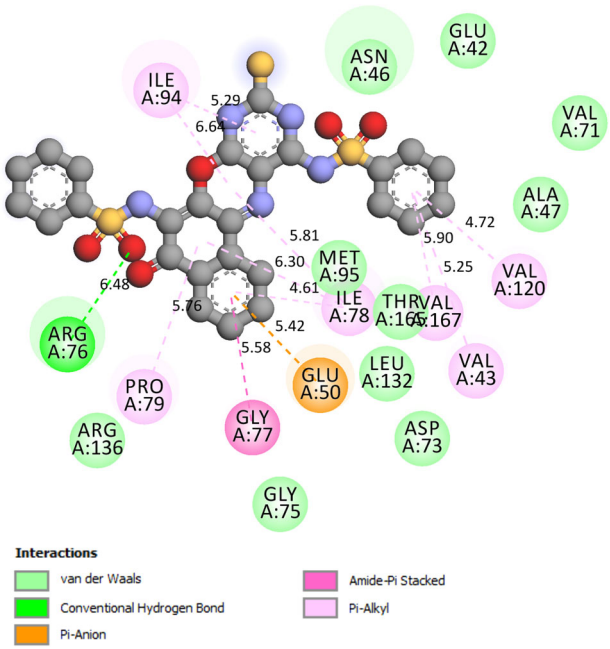


Fig. 7 Chemical interactions of 8a with intact DNA gyrase

one of the aromatic rings and GLY77. One of the O-atoms entered into hydrogen bonding interaction with ARG76. The details of the chemical interactions of 8a with DNA gyrase, including the distance of interaction (Å) are shown in Fig. 7. These various chemical interactions can explain the strong binding affinity of **8a** and its significant inhibitory effect on the protein. All the five 6-membered rings of compound **5b** were involved in the interactions between the compound and the mutated DNA gyrase (Fig. 6). The interactions were mainly pi-H interactions. The 6-membered ring interacted with the CB of ASN 46, CG1 of ILE 78, CD1 of ILE 78, CD of PRO 79 and CG1 of ILE 94. Though 5b showed no antimicrobial activity in vitro, it showed a promising binding affinity for the mutated DNA gyrase and may be a useful lead in the development of drugs to tackle multi drug resistant bacterial strains.

CONCLUSION

Newly synthesised aryl sulphonamidobenzo[a]phenozaxinones presented significant anti-microbial comparable to colymycin. In particular, 11-(4-methylphenylsulphonamido)-6-chloro-9-mercapto-8,10-diazabenzo[a]phenoxazin-5-one **7b** and 6,11-bis(phenylsulfonamido)-9-mercapto-8,10-diazabenzo[a]phenoxazin-5-one **8a** elicited broad spectra of activity against Gram positive and Gram negative bacteria. All the synthesized compounds have strong binding affinity with DNA gyrase; and therefore, can act as DNA gyrase inhibitors. They also have significant binding affinity for the mutated DNA gyrase. Therefore, they can be

used in the treatment of infections caused by resistant strains.

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