INDO AMERICAN JOURNAL OF PHARMACEUTICAL RESEARCH

NOVEL COMPOUNDS OF THIO-PYRIMIDINE ANTHRANILATE AND SULFONAMIDE ANTHRANILATE DERIVATIVES WITH ANTIMICROBIAL ACTIVITY AND IT'S SYNTHESIS

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ARTICLE INFO

Article history
Received 29/12/2018
Available online
05/01/2018

Keywords
Anthranilic Acid,
Thiopyrimidine Anthranilate,
Sulfonamide Anthranilate,
Antimicrobial.

ABSTRACT

Sulfonamide and Thio-pyrimidines derivatives are known for antibacterial activity and hence, in our research study, series of compounds were designed for the Thio-pyrimidine anthranilate and Sulphonamide anthranilate derivatives. Based on the N-terminal signal peptidase and C-terminal sorting signal of surface protein binding, Compound-1.4 (S-Allyl-Methyl ester), Compound 2.2 (S-Allyl-Amino-Acid), Compound-3.1 (Methyl-ester-Methyl-sulfonamide) & Compound-4.1 (Methyl-ester-Toluene-sulfonamide) were selected for our research as these compounds shown advantage in the binding. These four compounds were synthesized by chemical synthesis and characterized using structure elucidating techniques like NMR and Mass spectroscopy. These compounds were screened for the biological activity using Gram positive (Bacillus subtilis, Staphylococcus aureus) and Gram negative bacteria (Escherichia coli and Klebsiella Pneumoniae). In vitro Antibacterial Assay (Table-1) indicates that all the synthesized compounds (1.4, 2.2, 3.1 & 4.1) shows moderate to excellent activity data against all the tested Gram positive as well as Gram negative bacterial pathogens determined at concentration 10, 50, 100 and 200 µg/mL. From the activity data, it is concluded that the compound 2.2 & 4.1 are most active among all the tested compounds for the tested bacterial species, while compound 3.1 shows comparable activity compared to Chloramphenicol and Ciprofloxacin.

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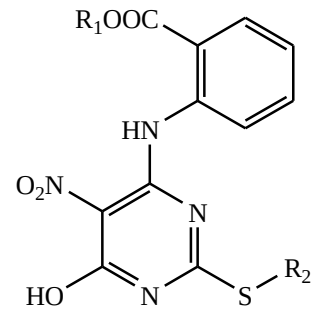
*Please cite this article in press as **Nitin A. Shimpi** et al. Novel Compounds of Thio-Pyrimidine Anthranilate and Sulfonamide Anthranilate Derivatives with Antimicrobial Activity and It's Synthesis. Indo American Journal of Pharmaceutical Research.2018:8(12).*

INTRODUCTION

Anthranilic acid is well known as o-amino benzoic acid (or) 2-amino benzoic acid. Anthranilic acid is also referred as Vitamin L1. Anthranilic acid and its esters are used in the preparation of perfumes to imitate Jasmine and Orange. Its derivatives are also used in the Pharmaceutical industry in a wide range of therapeutic categories [1]. Anthranilic acid derivatives are of the great importance in pharmaceutical industry because of their wide range of therapeutic activities. Anthranilic acid derivatives like Mefenamic acid, Tolfenamic acid, are Anti-inflammatory drugs [2]. Our core intention of research was to study and evaluate the biological activity for the Anthranilic acid derivative which has additional function group of sulfonamide and thiopyrimidine. Sulfonamide were the first antibiotics to be used systematically which led the path for the revolution in the antibiotics of medicine. Though the sulfonamides were primarily used for the antibiotic activity, however it is developed for the wide range of therapeutic categories like antifungal, antimicrobial [3], antimalarial, diuretics, COX-2 inhibitors [4], anti-diabetic, antiretroviral [5], antiarrhythmic [6][7] and antibiotic [8]. Pyrimidine is an aromatic heterocyclic organic compound which is a main fundamental component of RNA and DNA. The main nucleobases of pyrimidine derivatives are found in nucleic acid i.e. Cytosine, Thymine and Uracil. These nucleic acid plays an important role in showing antiretroviral (anti HIV) activity for many drugs [4][5][6]. Thio-pyrimidines derivatives also shows antibacterial activity [11][12].

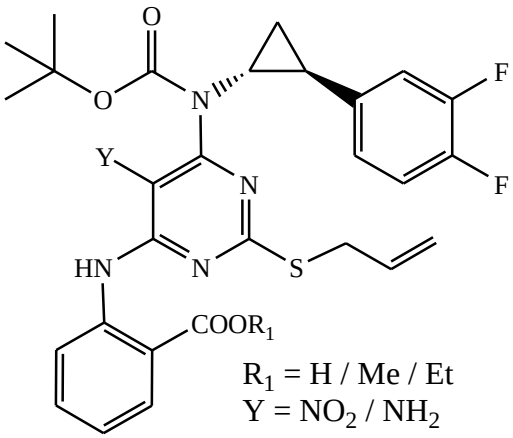
Worldwide there is a huge increase in the number of microbial infections because of antimicrobial resistance and there is continuously increasing the number of immune-compromised patients. Hence, there is an urgent need to search new antimicrobial compounds which will be effective against pathogenic micro-organics which have developed resistance to the antibiotics. We have reviewed for the activity of sulfonamide anthranilate and Thiopyrimidine anthranilate for antimicrobial activity. Some of the sulphonamide anthranilate shows antifungal and antimicrobial activity [9][10]. So in our research study series of compounds were designed for the Sulphonamide anthranilate and thio-pyrimidine anthranilate derivatives for the evaluation of antimicrobial activity. Following Lead compounds with the basic skeleton of pyrimidine-anthranilate and sulfonamide- anthranilate were designed. The present work deals with the synthesis and antimicrobial screening of novel derivatives.



Lead compound-1

R_1 = H / Alkyl

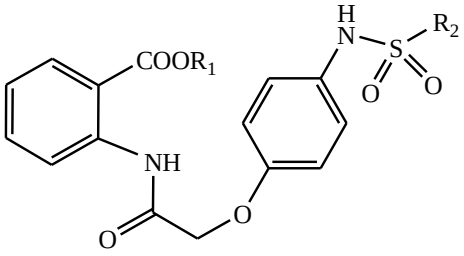
R_2 = Alkyl / allyl



R_1 = H / Me / Et

Y = NO_2 / NH_2

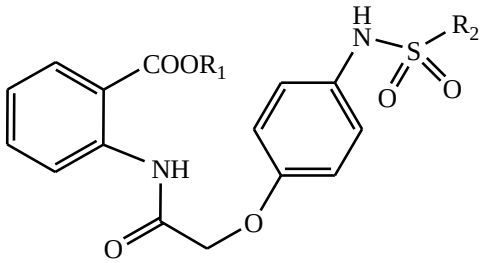
Lead compound-2



Lead compound-3

R_1 = H / Me

R_2 = Me / Et



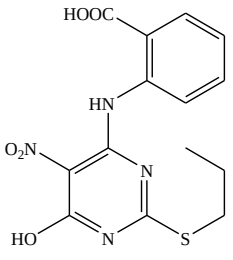
Lead compound-4

R_1 = H / Me

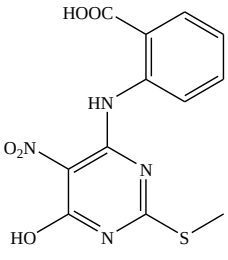
R_2 = Tolyl / Pheyl

MATERIAL AND METHODS

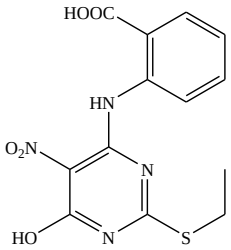
Based on the basic skeleton of Lead compound-1, following 4 compounds were designed and selected for the evaluation.



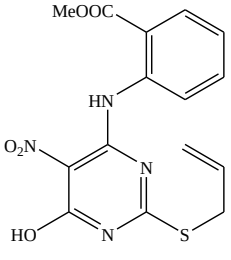
S-propyl-Acid
Compound-1.1



S-methyl-Acid
Compound-1.2

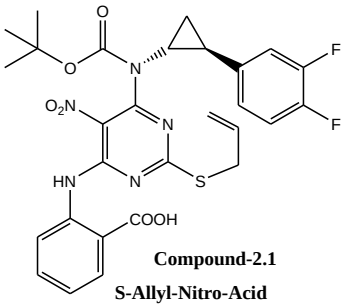


S-Ethyl-Acid
Compound-1.3

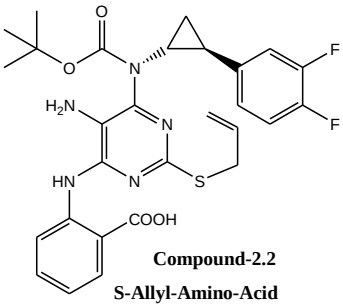


S-Allyl-Methyl ester
Compound-1.4

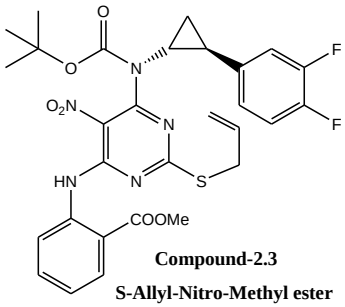
Similarly for the Lead compound-2, following 6 compounds were designed.



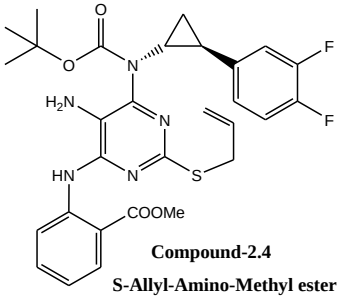
Compound-2.1
S-Allyl-Nitro-Acid



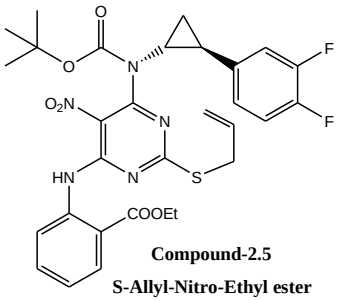
Compound-2.2
S-Allyl-Amino-Acid



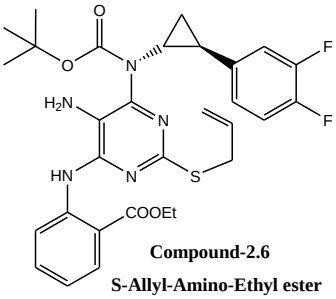
Compound-2.3
S-Allyl-Nitro-Methyl ester



Compound-2.4
S-Allyl-Amino-Methyl ester

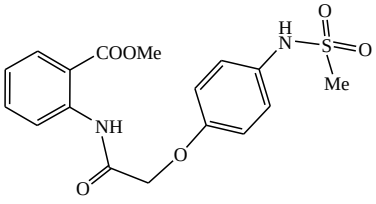


Compound-2.5
S-Allyl-Nitro-Ethyl ester

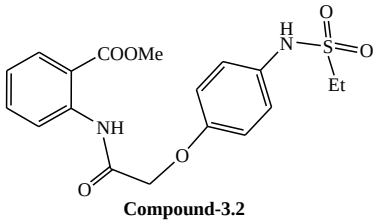


Compound-2.6
S-Allyl-Amino-Ethyl ester

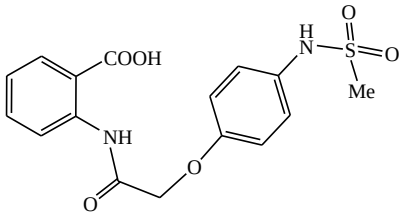
For Lead compound-3, following four compounds were designed and considered for the study.



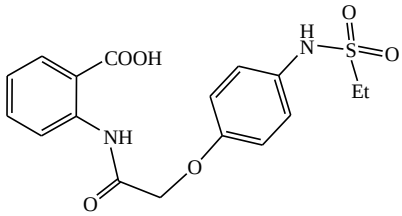
Compound-3.1
Methyl-ester-Methyl-Sulfonamide



Compound-3.2
Methyl-ester-Ethyl-Sulfonamide

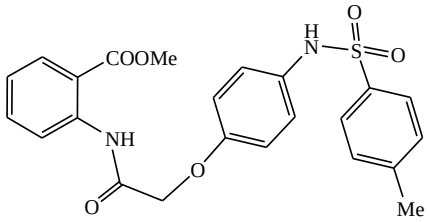


Compound-3.3
Acid-Methyl-Sulfonamide

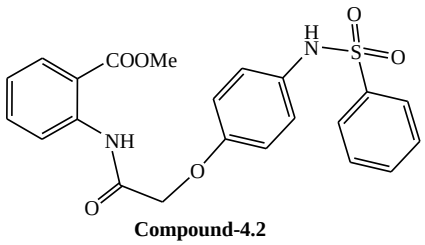


Compound-3.4
Acid-Ethyl-Sulfonamide

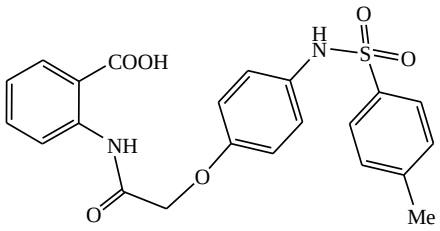
For Lead compound-4, following four compounds were designed by changing acid and ester functional group and considered for the study.



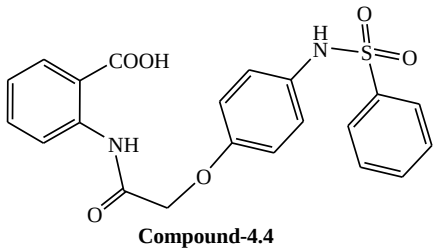
Compound-4.1
Methyl-ester-Toluene-Sulfonamide



Compound-4.2
Methyl-ester-Benzene-Sulfonamide



Compound-4.3
Acid-Toluene-Sulfonamide



Compound-4.4
Acid-Benzene-Sulfonamide

All the above structures were studied on the molecular modelling evaluation against the species of Bacillus anthracis and Styphallococcus aurus. Based on the N-terminal signal peptidase and C-terminal sorting signal of surface protein binding, Compound-1.4 (S-Allyl-Methyl ester), Compound 2.2 (S-Allyl-Amino-Acid), Compound-3.1 (Methyl-ester-Methyl-sulfonamide) and Compound-4.1 (Methyl-ester-Toluene-sulfonamide) were selected for our research as these compounds shown advantage in the binding over other structures.

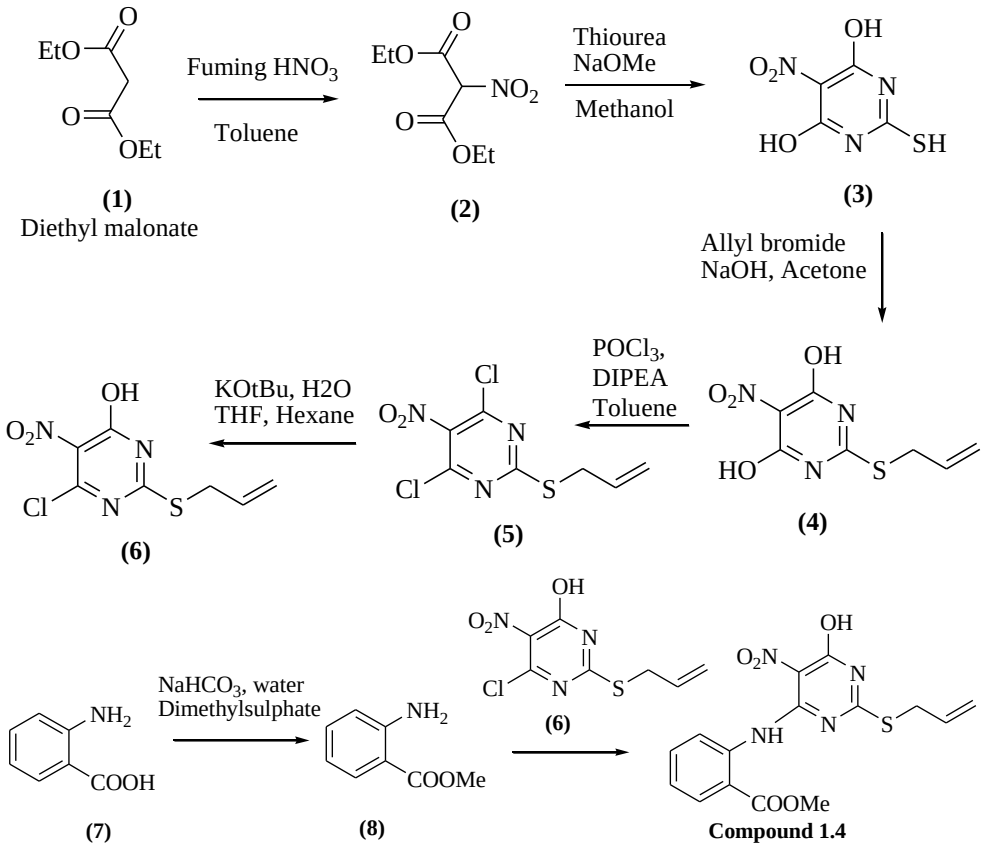
These four compounds were synthesized and characterized using structure elucidating techniques. These compounds were screened for the biological activity using Gram positive (*Bacillus subtilis, Staphylococcus aureus)* and Gram negative bacteria *(Escherichia coli and Klebsiella Pneumoniae).*

The IR spectra of compounds were recorded using a Perkin-Elmer spectrum one FT-IR spectrometer instrument by using 1% potassium bromide pellet technique. The ^{1}H and ^{13}C NMR spectra were recorded in DMSO-d_6 & $CDCl_3$ at 300 MHz & 75 MHz respectively on Bruker 300 MHz Avance NMR spectrometer using Tetramethylsilane as the internal standard. Mass spectra (MS) were recorded on Agilent 1100 Series LC-MSD-TRAP-SL instrument.

Reactions were monitored by thin layer chromatography on 0.2 mm silica gel 60 F_{254} (Merck) plates using UV light (254 and 366 nm) for detection. Common reagent-grade chemicals are commercially available and were used without further purification.

Synthesis of Compound-1.4 (S-Allyl-Methyl-Ester)

The compound 1.4 was synthesized in 7 steps starting with Diethyl malonate. Schematic representation of compound 1.4 synthesis is given below as per Schme-1. Compound (**5**) is prepared from Diethyl malonate as per the reported literature [16]



Scheme-1: Synthesis of Compound 1.4

Synthesis of Diethyl-2-nitromalonate (2) and 5-nitro-2-sulfanylpyrimidine-4, 6-diol (3)

Diethyl malonate **(1)** (100.0 g, 0.62 mol) was taken into flask and cooled to 10-15°C, fuming nitric acid (137.0 g, 2.17 mol) was added slowly by maintaining temperature. The reaction mass temperature was adjusted to 15-20°C. Reaction was monitored by TLC and after completion of the reaction, the reaction mixture was added to mixture of water (400 mL) and toluene (300 mL). Aqueous layer was then extracted with toluene (300 mL). Both the organic layers were combined and washed with water (500 mL) followed by 5% aqueous urea solution (500 mL) and Diethyl-2-nitromalonate (2) was extracted to aqueous layer using sodium carbonate solution (600 mL). pH of the aqueous layer was adjusted to 1.5 -2.5 with HCl and the compound (2) was extracted back with Toluene (500 mL). Organic layer was washed with by 5% aqueous urea solution (250 mL) followed by water (250 mL), then the organic layer was dried on sodium sulfate (100.0 g). Toluene layer containing compound (2) was taken in-situ for the next reaction.

In another flask sodium methoxide (78.0 g, 1.44 mol), Toluene (180 mL), Thiourea (50.0 g, 0.657 mol) was added followed by Toluene layer of compound (2). Reaction mixture was heated to 50-60°C & maintained for 3 hrs. After completion of reaction, water (270 mL) was added to the reaction mass and pH was adjusted to 2.0-3.0 with HCl. Reaction mass was cooled to room temperature and stirred for 2 hrs, the reaction mass was filtered and washed with water methanol mixture (1:1, 200 mL) and dried to get 105 g of 5-nitro-2-sulfanylpyrimidine-4, 6-diol (3) with 89% yield. Melting range 110-124°C; ^{1}H NMR (DMSO-d_6, 300 MHz) (δ, ppm): 11.15 (bs, 3H); ^{13}C NMR (DMSO-d_6,75 MHz) (δ, ppm): 115.2, 157, 174.7; Mass (m/z): 187.9 (M-H).

Synthesis of 5-nitro-2-allylthiopyrimidine-4,6-diol (4).

To the aqueous solution of Sodium hydroxide (75.0 g, 1.87 mol in 750 mL) 5-nitro-2-sulfanylpyrimidine-4, 6-diol (3) (100.0 g, 0.529 mol) was added and stirred till clear solution is observed. Allyl bromide (67.0 g, 0.55 mol) was added slowly at room temperature and maintained for 2 hrs. After the reaction completion, pH of was adjusted to 4.5-5.5 with aqueous HCl. Acetone (100 mL) was added to and stirred for 4-6 hrs. Product was filtered and washed with acetone: water mixture (1:1) (100 mL) and dried to get 105 g of 5-nitro-2-allylthiopyrimidine-4,6-diol (4) with the yield of 86.8%. Melting range 80-101°C; ^{1}H NMR (DMSO-d_6, 300 MHz) (δ, ppm): 3.69(d, 2H, *J*=6.9Hz), 5.81-5.94(m, 1H), 5.10 (d, 1H, *J*=10.2 Hz), 5.24-5.30(dd, 1H,*J*=16.8 Hz, 1.2 Hz), 11.21 (bs, 2H); ^{13}C NMR (DMSO-d_6,75 MHz) (δ, ppm):32.0, 117.8, 118.2, 133.5, 158.5, 163.7; Mass (m/z): 230 (M+H).

Synthesis of 4,6-dichloro-5-nitro-2-(prop-2-en-1-ylsulfanyl)pyrimidine (5).

Phosphorous oxychloride (350.0 g, 2.3 mol) was added to the solution of Toluene (400 mL) and 5-nitro-2-allylthiopyrimidine-4,6-diol **(4)** (100.0 g, 0.44 mol). Diisopropylethylamine (115.0 g, 0.89 mol) was also charged to it. The reaction mixture was heated up to 80-90°C and maintained for 4 hours. Reaction was monitored by TLC, reaction mass was distilled out under vacuum after completion of reaction followed by stripping one time with Toluene (100 mL). Toluene (500 mL) was added to the reaction mass, followed by addition of ice-water (600 mL) at 10-20°C. Layers were separated and aqueous layer was extracted with Toluene (200 mL). Both the Toluene layers were combined and washed with 5% Sodium bicarbonate solution (400 mL), followed by 20% aqueous sodium chloride solution (400 mL). Organic layer was stirred with silica (80.0 g) followed by charcoal treatment with activated carbon (5.0 g) at 50-55°C. Organic layer was then concentrated under reduced pressure at below 65°C to get 104 g of 4,6-dichloro-5-nitro-2-(prop-2-en-1-ylsulfanyl)pyrimidine **(5)** residue with the yield of 89.6%. ^{1}HNMR (DMSO-d_6, 300 MHz) (δ, ppm): 3.85(dd, 2H, *J*=6.9, 0.9Hz), 5.21-5.25(dd, 1H, *J*=9.9, 0.9Hz), 5.37-5.43(dd, 1H, *J*=16.8, 1.2Hz), 5.85-5.98(m, 1H); Mass (m/z): 266 (M+H).

Synthesis of 6-chloro-5-nitro-2-(prop-2-en-1-ylsulfanyl) pyrimidin-4-ol (6)

To the THF solvent (50 mL), Potassium tert-Butoxide (4.0g, 0.36 mol) was dissolved. 4,6-dichloro-5-nitro-2-(prop-2-en-1-ylsulfanyl)pyrimidine (5) (10.0 g, 0.37 mol) was dissolved in THF(50 mL) and slowly added to the above reaction mass by maintaining temperature below 15°C. Water (0.65g, 0.36 mol) was added to the reaction mass and stirred at 15-25°C for 4 hours. 20% aqueous Sodium chloride solution (100 mL) was added to the reaction mass and stirred for 30 minutes. Organic layer was separated and concentrated under vacuum to get reddish brown colored residue. Residue was stripped off two times with Hexanes (2x20 mL). Hexanes (100nl) was added to the reaction mass and stirred for 3-4 hours to get yellow colored 6 g solid material of 6-chloro-5-nitro-2-(prop-2-en-1-ylsulfanyl) pyrimidin-4-ol (6). ^{1}HNMR (DMSO-d_6, 300 MHz) (δ, ppm): 3.64(d, 2H, *J*=6.9 Hz), 5.05-5.09(dd, 1H, *J*=10.1, 0.8Hz), 5.22-5.28(dd, 1H, *J*=16.8, 1.5Hz), 5.83-5.97(m, 1H); ^{13}C NMR (DMSO-d_6,75 MHz) (δ, ppm): 32.9, 117.4, 132.2, 134.2, 147, 162.1, 168.3; Mass (m/z): 269.9 (M+Na).

Synthesis of Methyl anthranilate (8)

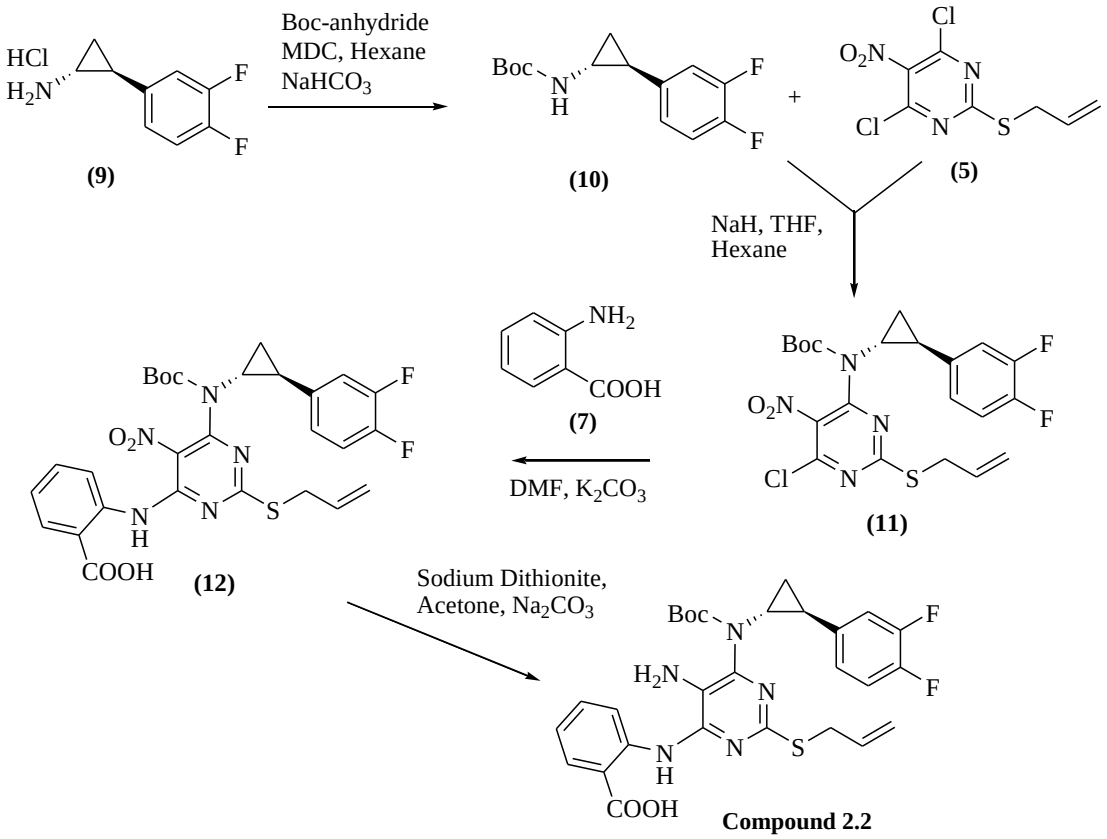
Anthranilic acid **(7)** (10.0g, 0.073 mol) was dissolved in aqueous Sodium bicarbonate solution (9.8g dissolved in 150 mL water), Dimethyl sulphate (11.0g, 0.87 mol) was added and stirred at room temperature for 3 hours. Ethyl acetate (100 mL) was charged to the reaction mass and product was extracted in Organic layer. Organic layer was concentrated to get Methyl anthranilate **(8)** ^{1}HNMR (DMSO-d_6, 300 MHz) (δ, ppm): 3.87(s, 3H), 5.69(bs, 2H), 6.61-6.67(m, 2H), 7.23-7.29(m, 1H), 7.86(dd, 1H, *J*=7.9, 0.9Hz).

Synthesis of Methyl, 2-(2-(allylthio)-6-hydroxy-5-nitropyrimidin-4-ylamino)benzoate (i.e.Compound 1.4):

To the DMF solvent (40 mL), 6-chloro-5-nitro-2-(prop-2-en-1-ylsulfanyl) pyrimidin-4-ol (6) (4.0g, 0.016 mol), Potassium Carbonate (8.0g, 0.057 mol) and Methyl anthranilate (8) (2.4g, 0.016 mol) was added and heated to 90-100°C and maintained for 12 hours. Reaction was monitored by TLC and cooled to room temperature after completion of reaction and water (120 mL) was added to it and stirred for 30 minutes and slurry filtered to get light yellow colored 6 g solid material of Methyl, 2-(2-(allylthio)-6-hydroxy-5-nitropyrimidin-4-ylamino)benzoate, compound 1.4. ^{1}HNMR (DMSO-d_6, 300 MHz) (δ, ppm): 3.62(d, 2H, *J*=6.9Hz), 3.8(s, 3H), 5.05(d, 1H, *J*=9.9 Hz), 5.13-5.19(dd, 1H, *J*=17.1, 1.5Hz), 5.82-5.93(m, 1H); ^{13}C NMR (DMSO-d_6, 75 MHz) (δ, ppm): 32.8, 52.2, 116.3, 116.7, 118.9, 122.4, 124.1, 130.3, 132.6, 134.8, 139.9, 154.9, 164.1, 166.8, 169.8; Mass (m/z): 363 (M+H), 385 (M+Na), 408(M+HCOOH).

Synthesis of Compound-2.2 (S-Allyl-Amino-Acid)

The Compound 2.2 was synthesized as per the Schme-2 given below. The input compound (1*R*, 2*S*)-2-(3,4-difluorophenyl)cyclopropanamine hydrochloride, i.e. compound (9) was synthesized following the reported literature [13][14][15]. Compound (11) was synthesized as per the reported literature [16].



Scheme-2: Synthesis of Compound-2.2.

Synthesis of *tert*-butyl[(1*R*,2*S*)-2-(3,4-difluorophenyl)cyclopropyl]carbamate (10)

(1*R*, 2*S*)-2-(3,4-difluorophenyl)cyclopropanamine hydrochloride, i.e. compound (9) (100.0 g, 0.48 mol) was dissolved completely in water (1000 mL). To this solution sodium bicarbonate (100.0 g, 1.19 mol), dichloromethane (700 mL) was added. Boc-anhydride (127.0 g, 0.58 mol) was added slowly at 25-30°C and maintained for 60 min. After reaction completion layers were separated and aqueous layer was extracted with dichloromethane (200 mL). Dichloromethane layers were combined and washed with 15% aqueous Sodium chloride solution (300 mL). Organic layer was concentrated at below 45°C to get residue. Hexanes (700 mL) was added to the residue and heated up to 60-70°C for 30 min. Reaction mass was then cooled to 25-35°C and maintained for 60 min, further it was cooled to 10-15°C for 2 hrs. Slurry was filtered and washed with chilled hexanes and dried under vacuum at 45-50°C to get 128 g of *tert*-butyl [(1*R*, 2*S*)-2-(3,4-difluorophenyl)cyclopropyl] carbamate (10), with the yield of 97.8%.

^{1}H NMR (DMSO-d_6, 300 MHz) (δ, ppm): 1.38 (s, 9H), 2.59 (bs, 1H), 1.08-1.16 (m, 2H), 1.88 – 1.94 (m, 1H), 6.96 (bs, 1H), 7.11-7.34 (m, 3H). ^{13}C NMR (DMSO-d_6,75 MHz) (δ, ppm):15.27, 23.49, 28.14, 33.23, 77.86, 114.62(d, *J*=17.2Hz), 116.94, 155.95, 122.62-122.73(dd, *J*=5.7, 3.0Hz), 139.47-139.60(dd, *J*=6.1, 3.5Hz), 146.92-149.31(dd, *J*=241.2, 12.6Hz), 147.59-151.00(dd, *J*=243.0, 12.6Hz); Mass(m/z): 292 (M+Na).

***tert*-butyl[6-chloro-5-nitro-2-(prop-2-en-1-ylsulfanyl)pyrimidin-4-yl][(1*R*,2*S*)-2-(3,4-difluorophenyl)cyclopropyl]carbamate (11)**.

To a Sodium hydride (25 g, 0.63mol), Tetrahydrofuran (500 mL) was added under nitrogen atmosphere and cooled to -10°C. Solution of compound (10) [(96.0 g, 0.36mol) was dissolved in Tetrahydrofuran (300 mL)] was added slowly to the pre-cooled sodium hydride solution at -7 to -13°C, temperature was adjusted to 20°C and maintained for 120 min. In another flask 4,6-dichloro-5-nitro-2-(prop-2-en-1-ylsulfanyl)pyrimidine (5) (100.0 g, 0.37mol) was dissolved in Tetrahydrofuran (500 mL) under nitrogen atmosphere and cooled to -20°C. Sodium hydride solution was added to this solution by maintaining temperature at -10 to -20°C, after completion of the reaction, temperature was raised and 20% Sodium chloride solution (500 mL) was added. Organic layer was separated and concentrated by vacuum distillation and stripped off with hexanes (200 mL) to get residue. Hexanes (1000 mL) was charged to the residue and stirred at room temperature for 3-4 hr. The obtained slurry was filtered and washed with hexanes (100 mL). Filtrate was then concentrated under vacuum at below 55°C to get 182 g residue of *tert*-butyl[6-chloro-5-nitro-2-(prop-2-en-1-ylsulfanyl)pyrimidin-4-yl][(1*R*,2*S*)-2-(3,4-difluorophenyl)cyclopropyl] carbamate (11), yield 98.4%.

^{1}H NMR (DMSO-d_6, 300 MHz) (δ, ppm): 1.32-1.36 (m, 1H), 1.48-1.57 (m, 1H), 1.36 (s, 9H), 2.25-2.31 (m, 1H), 3.21-3.26 (m, 1H), 3.63-3.80 (m, 2H), 5.14 (d, 1H, *J*=10.2 Hz), 5.33 (d, 2H, *J*=16.8 Hz), 5.68-5.93 (m, 2H), 7.05-7.08(m, 1H), 7.27-7.38 (m, 2H); ^{13}CNMR (DMSO-d_6,75 MHz) (δ, ppm): 18.1, 25.71, 27.35, 33.71, 39.12, 83.89, 115.18(d, *J*=17.4Hz), 116.99(d, *J*=16.8 Hz), 118.89, 122.95-123.07(dd, *J*=6.0, 3.2Hz), 137.95-138.09(dd, *J*=6.3, 3.5Hz), 146.27-149.66(dd, *J*=241.9, 12.6Hz), 147.56-150.97(dd, *J*=243.2, 12.5Hz), 132.30, 134.65, 151.75, 152.56, 155.51, 172.05; Mass (m/z): 498.9 (M+H).

Synthesis of 2-{[6-{(*tert*-butoxycarbonyl)[(1*R*,2*S*)-2-(3,4-difluorophenyl) cyclopropyl] amino}-5-nitro-2-(prop-2-en-1-ylsulfanyl)pyrimidin-4-yl]amino} benzoic acid (12).

tert-butyl[6-chloro-5-nitro-2-(prop-2-en-1-ylsulfanyl)pyrimidin-4-yl][(1*R*,2*S*)-2-(3,4-difluorophenyl) cyclopropyl]carbamate (11) (4.8 g, 0.0096 mol) residue was dissolved in Dimethyl formamide (40 mL) and Anthranilic acid (7) (1.32g, 0.0096) and Potassium carbonate (5.6g, 0.0405 mol) was added along with Copper(I) iodide (10mg) and the reaction mass was heated to 100-105°C and maintained for 10 hours. Reaction was monitored by TLC. After reaction completion, reaction mass was quenched in water (200 mL) and the product was extracted with Ethyl acetate (100 mL) Aqueous layer was back extracted with Ethyl acetate (30 mL). Both the organic layers were mixed and washed with 10% aqueous Sodium chloride solution (70 mL). Organic layer was then concentrated under vacuum to get residue 5.1 g of 2-{[6-{(tert-butoxycarbonyl)[(1*R*,2*S*)-2-(3,4-difluorophenyl)cyclopropyl] amino}-5-nitro-2-(prop-2-en-1-ylsulfanyl)pyrimidin-4-yl]amino} benzoic acid (12), product confirmation was done by mass and the proceeded to next step without purification. Mass (m/z): 600 (M+H).

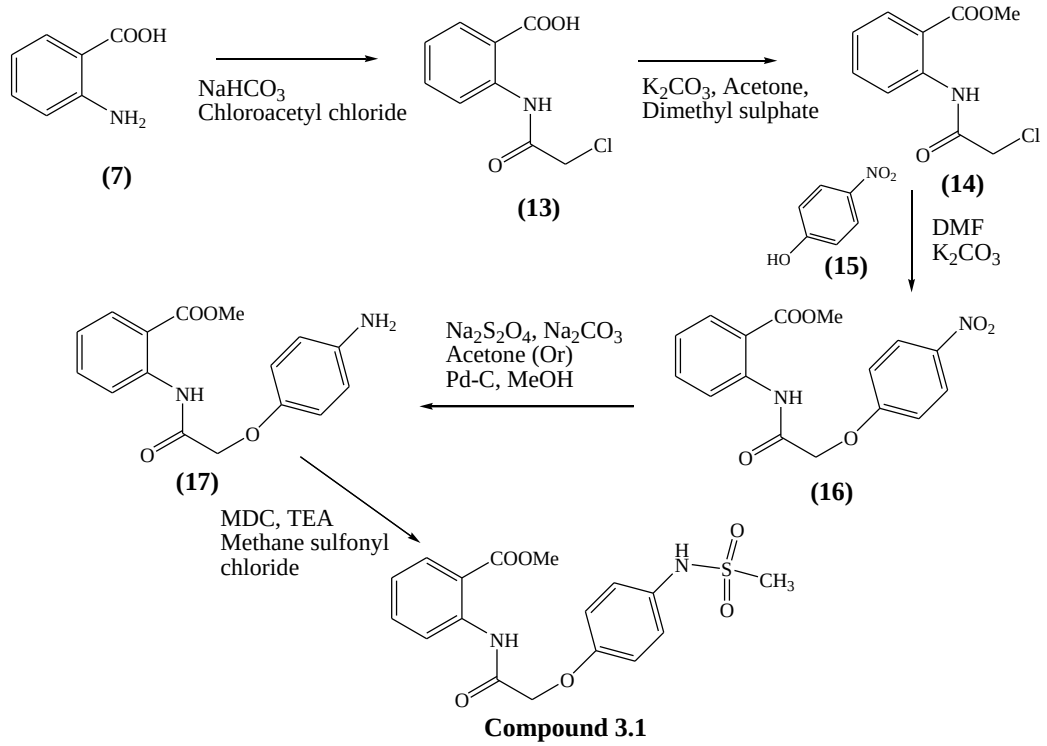
Synthesis of 2-{[5-amino-6-{(tert-butoxycarbonyl)[(1*R*,2*S*)-2-(3,4-difluorophenyl) cyclopropyl]amino}-2-(prop-2-en-1-ylsulfanyl)pyrimidin-4-yl]amino}benzoic acid (2.2).

Compound (12) (5.0 g, 0.0083 mol) was dissolved in Acetone (50 mL) and aqueous solution of Sodium carbonate (4.42 g in 20 mL water) was added to the reaction mass at room temperature. Sodium dithionite (7.3g, 0.0417 mol) was added in 5 lots with the interval of 5 minutes and stirred for 2 hours at 35-40°C. Reaction was monitored by TLC and reaction mass was filtered after completion of reaction. Filtrate was concentrated under vacuum by removing acetone. Water (100 mL) and Ethyl acetate (100 mL) was added to reaction mass. Organic layer was separated and washed with 20% aqueous sodium chloride solution. Organic layer was dried on sodium sulphate and concentrated to get residue which on trituration with Isopropyl ether (30 mL) gave solid material, this material was purified by Column chromatography using Ethyl acetate and Hexane solvent to get 4.1 g of 2-{[5-amino-6-{(tert-butoxycarbonyl)[(1*R*,2*S*)-2-(3,4-difluorophenyl)cyclopropyl]amino}-2-(prop-2-en-1-ylsulfanyl) pyrimidin-4-yl]amino}benzoic acid (2.2).

^{1}H NMR(DMSO-d_6,300 MHz) (δ, ppm): 1.09-1.30(m, 2H), 1.37(s, 9H), 2.33(m, 1H), 3.13-3.18(m, 1H), 3.69(d, 1H, *J*=6 Hz), 4.44(bs, 2H), 5.01(d, 1H, *J*=9 Hz), 5.18(d, 1H, *J*=18.0Hz), 5.87-5.96(m, 1H), 7.03-7.10(m, 2H), 7.16-7.37(m, 2H), 7.59(t, 1H), 8.01(d, 1H, *J*=9.0 Hz), 8.60(d, 1H, *J*=9.0 Hz), 11.12(s, 1H); Mass (m/z): 570 (M+H).

Synthesis of Compound-3.1 (Methyl-Ester-Methyl-Sulfonamide)

The compound 3.1 was synthesized from Anthranilic acid in 5 steps as per Schme-3 given below. Compound (**16**) was synthesized from Anthranilic acid (**7**) in the similar manner to that of reported literature [17].



Scheme-3: Synthesis of compound 3.1.

Synthesis of 2-(2-chloroacetamido)benzoic acid (13).

Sodium bicarbonate (27.5 g, 0.328 mol) was taken into flask and dissolved in water (600 mL). Anthranilic acid **(7)** (30.0 g, 0.219 mol) was added slowly and stirred till complete dissolution is observed at room temperature. Chloroacetyl chloride (29.6 g, 0.262 mol) was slowly added to the solution, material was precipitated during the addition, reaction mass was stirred for 2 hrs and the progress of reaction was monitored by TLC, after reaction completion, reaction slurry was filtered and washed with water (100 mL).Filtered material was dried to get 46.1 g of 2-(2-chloroacetamido)benzoic acid **(13)** with 98% yield. Melting range 182-184°C;
^{1}H NMR ($CDCl_3$, 300 MHz) (δ, ppm): 4.23(s, 2H), 7.19(t, 1H, *J*=6.0Hz), 7.62(dt, 1H, *J*=6.0, 1.2Hz), 8.15(dd, 1H, *J*=6.0, 1.2Hz), 8.73(d, 1H, *J*=6.0Hz), 11.77(bs, 1H). ^{13}C NMR (DMSO-d6, 75 MHz) (δ, ppm): 43.4, 116.8, 119.8, 123.5, 131.2, 134.2, 139.9, 165.2, 169.3; Mass (m/z): 213.9 (M+H).

Synthesis of Methyl, 2-[(2-chloroacetyl)amino]benzoate (14).

2-(2-chloroacetamido)benzoic acid (13) (30.0 g, 0.14 mol) was charged to the flask along with Acetone (300 mL), Potassium carbonate (20.3 g, 0.148 mol) was added and stirred for 15 minutes. Dimethyl sulphate DMS (26.6 g, 0.211 mol) was added slowly drop wise to the reaction mass at room temperature allowing it to raise to 38°C and the reaction mass was maintained for 2 hrs. Progress of reaction was monitored by TLC, after completion of the reaction, acetone was distilled out under vacuum, water (150 mL) Ethyl acetate (150 mL) was charged to the reaction mass and layers were separated, aqueous layer was back extracted with ethyl acetate (60 mL). Both the Ethyl acetate layers were mixed and concentrated under vacuum at 45-55°C, Hexane (150 mL) was charged to the residue and gradually cooled to 10-15°Cand filtered off, washed with chilled Hexanes (30 mL). Filtered material was dried under vacuum at 40-45°Cto get 31.4 g of Methyl, 2-[(2-chloroacetyl)amino]benzoate (14) with 98.2% yield. Melting range 96-98.5°C.
^{1}H NMR ($CDCl_3$, 300 MHz) (δ, ppm): 3.96(s, 3H), 4.21(s, 2H), 7.16(dt, 1H, *J*=8.1, 0.6Hz), 7.57(dt, 1H, *J*=8.7, 1.8Hz), 8.06(dt, 1H, *J*=8.1, 1.5Hz), 8.69(d, 1H, *J*=8.7Hz), 11.87(bs, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz) (δ, ppm): 43.2, 52.5, 115.8, 120.4, 123.4, 130.9, 134.5, 140.2, 165.2, 168.2; Mass (m/z): 228 (M+H), 250(M+Na).

Synthesis of Methyl, 2-(2-(4-nitrophenoxy)acetamido)benzoate (16).

To a stirred solution of DMF (100 mL) and 4-nitrophenol (15) (12.8 g, 0.092 mol), Potassium carbonate (18.2 g, 0.132 mol) was added and heated to 55-60°C. Methyl, 2-[(2-chloroacetyl)amino]benzoate (14) (20.0 g, 0.088 mol) was dissolved in DMF (100 mL) and slowly added to the above reaction mass at 55-60°C and then maintained for 6 hours. Reaction was monitored by TLC and after reaction completion, temperature was reduced to 30°C. In another flask water (1200 mL) was taken and the above reaction mass was slowly quenched to it. Reaction mass was cooled to 30°C and maintained for 30 minutes. Precipitated material was filtered off and washed with water (50 mL) and material was dried under reduced pressure at below 55 °C to get lemon colored powder 27.2 g of Methyl, 2-(2-(4-nitrophenoxy)acetamido)benzoate (16) with the yield of 94%.
^{1}H NMR (DMSO-d_6, 300 MHz) (δ, ppm): 3.90(s, 3H), 4.95(s, 2H), 7.24(t, 1H, *J*=6.0 Hz), 7.43(d, 1H, *J*=6.0Hz), 7.68(t, 1H, *J*=6 Hz), 8.00(dd, 1H, *J*=6.0, 2.0Hz), 8.28(dd, 2H, *J*=6.0, 1.1Hz), 8.55(dd, 1H, *J*=9.0, 0.45Hz), 11.64(bs, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz) (δ, ppm): 52.6, 67.6, 115.5, 116.2, 120.2, 123.5, 125.9, 130.8, 134.5, 139.3, 141.7, 162.1, 166.1, 167.6; Mass (m/z): 331 (M+H), 353 (M+Na).

Synthesis of Methyl, 2-(2-(4-aminophenoxy)acetamido)benzoate (17).

Methyl, 2-(2-(4-nitrophenoxy)acetamido)benzoate (16) (15.0 g, 0.045 mol) was dissolved in Acetone (250 mL), aqueous solution of Sodium carbonate (24 g, 0.227 mol in 200 mL water) was added to it. Sodium dithionite (40.0 g, 0.227 mol) was added in 5 lots at 35-40°C. Reaction was maintained for 2 hours. Reaction was monitored by TLC and after reaction completion, water (500 mL) and Ethyl acetate (500 mL) was charged to the reaction mass and stirred for 30 minutes. Organic layer was separated and washed with 10% aqueous Sodium chloride solution. Organic layer was concentrated under vacuum and striped off with Di-isopropyl ether and triturated with Di-isopropyl ether (100 mL) to get 13.2 g of Methyl, 2-(2-(4-aminophenoxy)acetamido) benzoate (17) with the yield of 97%.
^{1}HNMR ($CDCl_3$, 300 MHz) (δ, ppm): 3.33(s, 2H), 3.93(s, 3H), 4.56(s, 2H), 6.65(d, 2H, *J*=9.0 Hz), 6.90(d, 2H, *J*=9.0 Hz), 7.12(t, 1H, *J*=9.0 Hz), 7.55(t, 1H, *J*=6.0 Hz), 8.03(dd, 1H, *J*=6.0, 1.0 Hz), 8.77(dd, 1H, *J*=9.0, 1.1 Hz), 12.01(s, 1H); ^{13}C NMR ($CDCl_3$, 75 MHz) (δ, ppm): 52.2, 68.6, 115.8, 115.9, 116.3, 120.4, 123.0, 130.8, 134.3, 140.2, 141.0, 150.5, 167.9; Mass (m/z): 301 (M+H).

Methyl, 2-(2-(4-aminophenoxy)acetamido)benzoate (17) was also synthesized by catalytic hydrogenation using Palladium on Carbon catalyst and the experimental procedure is given below.

Synthesis of Methyl, 2-(2-(4-aminophenoxy)acetamido)benzoate (17).

Methyl, 2-(2-(4-nitrophenoxy)acetamido)benzoate (16) (20.0 g, 0.0606 mol) was dissolved in Methanol (400 mL), Palladium on Carbon (1 g) was added to the solution and the reaction mass was hydrogenated at 5-6 Kg hydrogen pressure at 35-40°C. Reaction was maintained at 35-40°C with 5-6 Kg hydrogen pressure for 6 hours. Reaction was monitored by TLC and after reaction completion, reaction mass was filtered and filtrate was concentrated under vacuum to get residue and striped off with Di-isopropyl ether and finally triturated with Di-isopropyl ether (100 mL) to get 17.6 g of Methyl, 2-(2-(4-aminophenoxy)acetamido)benzoate (17) as a base and HCl salt was prepared by dissolving in Ethyl acetate and adding HCl to get HCl salt of Methyl, 2-(2-(4-aminophenoxy)acetamido)benzoate (17).
^{1}H NMR (DMSO-d_6, 300 MHz) (δ, ppm): 3.90(s, 3H), 4.78(s, 2H), 7.20(d, 2H, *J*=6.0 Hz), 7.22(t, 1H, *J*=9.0 Hz), 7.41(d, 2H, *J*=6.0 Hz), 7.66(t, 1H, *J*=9.0 Hz), 7.99(d, 1H, *J*=6.0 Hz), 8.58(d, 1H, *J*=9.0 Hz), 10.38(bs, 3H), 11.66(s, 1H). ^{13}C NMR (DMSO-d_6, 75 MHz) (δ, ppm): 52.0, 67.0, 115.3, 115.5, 119.5, 122.8, 124.4, 124.8, 130.2, 133.9, 138.9, 155.9, 166.2, 166.9; Mass (m/z): 301 (M+H).

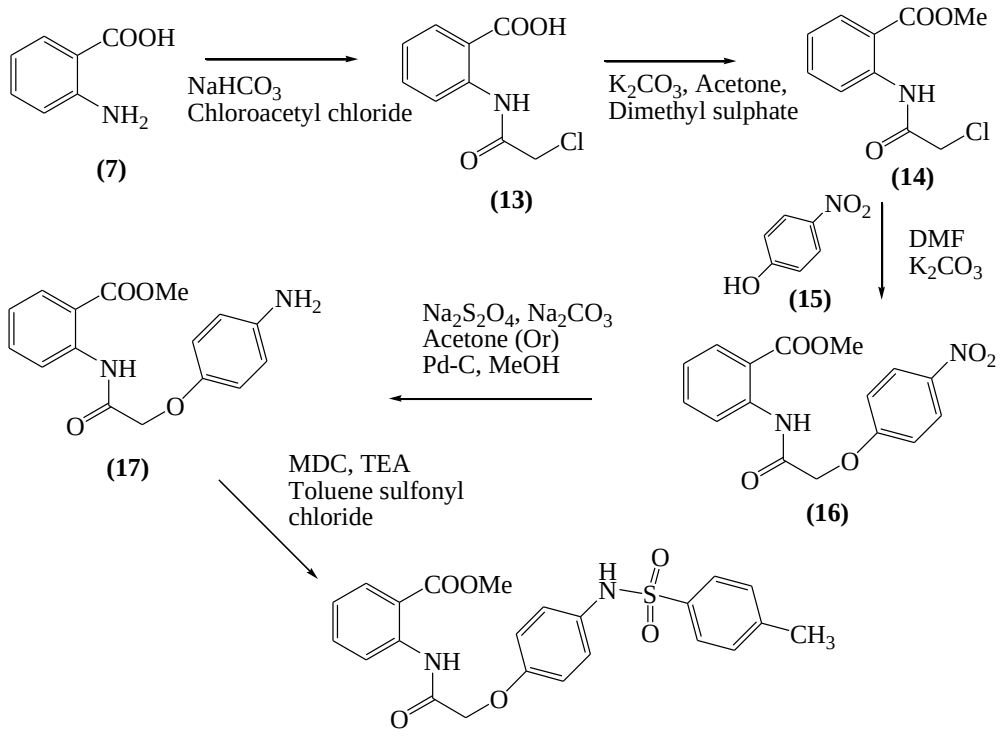
Synthesis of Methyl, 2-[({4-[(methylsulfonyl)amino]phenoxy}acetyl)amino] benzoate (Compound 3.1).

Methyl, 2-(2-(4-aminophenoxy)acetamido)benzoate **(17)** (1.0 g, 0.003 mol) was dissolved in Dichloromethane (25 mL), Triethylamine (0.40 g, 0.004 mol) was added to the solution and the reaction mass was cooled to 5-8°C, Methane sulfonyl chloride (0.40g, 0.0035 mol) was diluted with Dichloromethane (5 mL) and slowly added to the reaction mass. Reaction temperature was brought to room temperature and maintained at 30-35°C for 1 hour. Reaction was monitored by TLC, unreacted starting material was observed and hence again Triethylamine (0.1g) was added to the reaction mass and stirred for 15 min. Methanesulfonyl chloride (0.1g) was added to the reaction mass and maintaining for 30 minutes. Reaction completion was monitored by TLC. Water (40 mL) and Dichloromethane (20 mL) was added to the reaction mass. Organic layer was separated and washed with 5% Sodium bicarbonate solution (20 mL). Organic layer was concentrated and Methyl tert-Butyl ether (MTBE) 20 mL was added to the reaction mass and stirred for 45 minutes. Solid material was filtered and dried to get 1.1 g of Compound 3.1.

^{1}H NMR (DMSO-d_6, 300 MHz) (δ, ppm): 2.87(s, 3H), 3.89(s, 3H), 4.72(s, 2H), 7.07-7.26(m, 5H), 7.66(dt, 1H), 8.0-8.02(dd, 2H, *J*=8.1, 1.5Hz), 8.60-8.63(dd, 1H, *J*=8.4, 0.9Hz), 9.47(s, 1H), 11.68(s, 1H); Mass (m/z): 379 (M+H), 401 (M+Na).

Synthesis of Compound-4.1 (Methyl-Ester-Toluene-Sulfonamide)

The compound 4.1 was synthesized from Methyl, 2-(2-(4-aminophenoxy) acetamido)benzoate (17) in a single step by reacting with Benzene sulfonyl chloride in the presence of Triethyl amine, as per the Schme-4 given below. The compound (17) was synthesized in the similar procedure which was adapted for the synthesis of compound (3.1).



Compound 4.1

Scheme-4: Synthesis of Compound 4.1.

Synthesis of Methyl, 2-{[(4-{[(4-methylphenyl)sulfonyl]amino}phenoxy)acetyl] amino}benzoate (4.1).

Methyl, 2-(2-(4-aminophenoxy)acetamido)benzoate (17) (1.0 g, 0.0033 mol) was dissolved in Dichloromethane (25 mL), Triethylamine (0.51 g, 0.005 mol) was added to the solution and the reaction mass was cooled to 20-25°C, Toluene sulfonyl chloride (0.70g, 0.0037 mol) was diluted with Tetrahydrofuran (5 mL) and slowly added to the reaction mass. Reaction temperature was brought to room temperature and maintained at 30-35°C for 2 hour. Reaction was monitored by TLC, unreacted starting material was observed and hence again Triethylamine (0.2g) was added to the reaction mass and stirred for 15 min. Toluene sulfonylchloride (0.1g) was added to the reaction mass and maintaining for 12 hours. Reaction completion was monitored by TLC. Water (40 mL) was added to the reaction mass. Organic layer was separated and washed with 10% Sodium carbonate solution (40 mL). Organic layer was then washed with water (40 mL) and concentrated to residue. Di-isopropyl ether (30 mL) was added to the reaction mass and stirred for 45 minutes. Solid material was filtered and dried to get 1.2 g of Methyl, 2-{[(4-{[(4-methylphenyl)sulfonyl]amino}phenoxy) acetyl]amino}benzoate (4.1).

^{1}H NMR (DMSO-d_6, 300 MHz) (δ, ppm): 2.32(s, 3H), 3.87(s, 3H), 4.66 (s, 2H), 6.96-7.06(m, 4H), 7.23(dt, 1H), 7.30(d, 2H, *J*=6 Hz), 7.56(d, 2H, *J*=9 Hz), 7.66(dt, 1H), 7.99-8.02(dd, 2H, *J*=8.1, 1.5 Hz), 8.58-8.62(dd, 1H, *J*=9.0, 1.5Hz), 9.95(s, 1H), 11.63(s, 1H). ^{13}C NMR (DMSO-d_6, 75 MHz) (δ, ppm): 20.9, 52.5, 67.5, 115.4, 115.9, 120.0, 123.0, 123.3, 126.7, 129.5, 130.8, 131.5, 134.5, 136.6, 139.5, 143.0, 154.1, 167.0, 167.4; Mass (m/z): 454.7 (M+H), 476.7(M+Na).

RESULTS AND DISCUSSION

As the basic pyrimidine and sulfonamide moiety is showing majorly antimicrobial activity and hence the derivatives of Pyrimidine-anthranilate and Sulfonamide-anthranilate (i.e. Compound 1.4, Compound 2.2, Compound 3.1 and Compound 4.1) were taken for the testing of Antimicrobial activity.

The antimicrobial activity of the synthesized compounds were studied, systematically against four different strains of bacteria (Gram positive and Gram negative) by agar diffusion method. Generally, the antibacterial activity of compound is expressed internal of its ability to inhibit the growth of bacteria in nutrient broth or agar, the bacteria inhibit can be measured by two methods. Serial dilution method and other is diffusion method. The serial dilution method is useful for qualitative detection tests and also for evaluation of large number of compounds [18]. Therefore in this investigation diffusion method is employed.

The agar diffusion method is of three types i) cup plate method (disc method) ii) filter method iii) gradient plate method. The specific method used in our investigation was cup plate method, involving cup standard diameter, the nutrient agar medium and containing standard bacterial inoculums. The tests compounds were introduced into the cups and the diameter of zone of inhibitions was measured. All test compounds were evaluated for antimicrobial activity against *Staphylococcus aureus* & *Bacillus subtilis* (Gram positive), *Escherichia coli, & Klebsiella Pneumoniae* (Gram negative) following agar diffusion method.

The organisms were subculture using nutrient agar medium. The tubes containing sterilized medium were inoculated with respective bacterial strains. After incubation at 37±1°C for 24 hrs they were store in refrigerator. Thus the stock cultured was maintained. Bacterial inoculums were prepared by transferring a loop full of stock culture to nutrient broth (100 mL) in a clean and sterilized conical flask (250 mL). The flasks were incubated at 37± 1°C for 18h before the experimentation. The solution of the test compound prepared by dissolving 10 µg, 50 µg, 100 µg and 200 µg of each in DMSO (AR grade) A reference standard for Gram positive and Gram negative bacteria were made by dissolving accurately weighed quantity of Ciprofloxacin and chloramphenicol respective solution in DMSO separately.

The nutrient agar medium was sterilized by autoclave at 120°C (15lb/sq.inch). The petri-plates, tubes and flasks plugged with cotton were sterilized in hot air oven at 160°C for an hour. In to each sterilized petri-plate (10 cm diameter) about 30 mL each of molten nutrient bacteria (6 mL of inoculums to 300 mL of nutrient agar medium) was transferred aseptically. The plates were left at room temperature for to allow the solidification. In each plate four cups were made with a sterile borer. Then 0.1 mL of the test solution was added to the cups, aseptically and labelled accordingly. The plates were kept undisturbed for at least 2hr at room temp to allow the diffusion of solution properly in to nutrient agar medium. After incubation of plate at 37±1°C for 24 hours the diameter of the zone of inhibition was measured with the help of antibiotic zone reader. All experiment was carried out triplicate. Simultaneous controls were maintained employing 0.1 mL of DMSO to observe the solvent effect.

The results of the study are presented in the Table 1 as given below.

Table-1. Antimicrobial activity of Anthranilic acid derivatives (Compound 1.4, compound 2.2, compound 3.1 & compound 4.1).

Compound No	Conc. (µg/disc)	Zone of inhibition (mm)			
		A	B	C	D
Compound 1.4	10	3	2	3	1
	50	7	6	6	5
	100	9	8	7	6
	200	12	13	12	10
Compound 2.2	10	16	18	14	13
	50	32	31	29	30
	100	38	37	36	37
	200	43	41	40	39
Compound 3.1	10	19	18	14	13
	50	31	32	32	30
	100	34	36	31	34
	200	36	38	33	35
Compound 4.1	10	18	12	14	12
	50	28	29	31	28
	100	31	32	37	34
	200	34	35	38	36
Ciprofloxacin	10	24	26	23	25
	50	30	32	37	35
	100	34	36	40	37
	200	38	42	45	42
Chloramphenicol	10	26	28	25	26
	50	30	32	31	34
	100	35	38	40	42
	200	39	41	44	45

Where A = *Staphylococcus aureus*, B = *Bacillus subtilis*, C = *Escherichia coli*, D = *Klebsiella Pneumoniae*.

In vitro Antibacterial Assay (Table-1) indicates that all the synthesized compounds (1.4, 2.2, 3.1 & 4.1) shows moderate to excellent activity data against all the tested Gram positive as well as Gram negative bacterial pathogens determined at concentration 10, 50, 100 and 200 µg/mL. From the above activity data, it is observed that the compound 2.2 & 4.1 are most active among all the tested compounds against tested bacterial species, while compound 3.1 shows comparable activity and Compound 1.4 shows less activity compared to Chloramphenicol and Ciprofloxacin.

CONCLUSION

In conclusion, we have described synthetic method of novel derivatives of Anthranilic acid (Compound 1.4, Compound 2.2, Compound 3.1 and Compound 4.1). Antimicrobial activity of these synthesized compounds. All the synthesized compounds were characterized and confirmed by spectral and analytical methods. From the antimicrobial study, three compounds 2.2, 3.1 and 4.1 demonstrated potent inhibition against all the Antibacterial strains. We conclude that the synthesized compounds Methyl, 2-(2-(allylthio)-6-hydroxy-5-nitropyrimidin-4-ylamino)benzoate, (i.e. Compound 1.4), 2-{[5-amino-6-{(tert-butoxycarbonyl)[(1*R*,2*S*)-2-(3,4-difluorophenyl) cyclopropyl]amino}-2-(prop-2-en-1-ylsulfanyl)pyrimidin-4-yl]amino}benzoic acid (i.e. Compound 2.2), Methyl, 2-[({4-[(methylsulfonyl)amino]phenoxy}acetyl)amino] benzoate (i.e. Compound 3.1) and Methyl, 2-{[(4-{[(4-methylphenyl)sulfonyl]amino}phenoxy)acetyl]amino}benzoate (i.e. Compound 4.1), exhibited good to moderate antimicrobial activity. The importance of such work lies in the possibility that the synthesized compounds might be more efficacious drugs against other bacterial species which could be helpful in designing potent antibacterial compounds for therapeutic use hence recommend future research.

ACKNOWLEDGMENTS

The author express special thanks to Mylan laboratories for analytical support & Dr. Shital Zambad for his extended guidance and support.

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